The influence of phenotypic and genotypic factors on the colour of lamb meat during retail display

This thesis is presented for the degree of

Doctor of Philosophy of Murdoch University

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B.V.M.S. (Veterinary Medicine and Surgery)

Hons

August 2017

Murdoch
UNIVERSITY

Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Summary

The colour of lamb meat on retail display is critical to consumer appeal. Consumers demand a bright red colour in lamb meat and associate dark, pale or browned meat with a lack of freshness and quality. Retailers are forces to downgrade or discount discoloured meat to prevent consumer rejection. Fresh lamb meat may be discoloured due to poor colour development with blooming, or due to browning that develops with time on retail display. The quantity of lamb meat with poor bloomed colour is unknown because lamb carcasses are not routinely graded for meat colour in Australia. The rapid browning of overwrapped lamb meat limits its retail display to only around 2 days. Retailers frequently discount lamb meat to ensure its rapid sale prior to the onset of browning and to thereby limit the economic losses associated with discounting, downgrading or wasting browned meat.

Better understanding is required of the factors influencing bloomed colour of lamb meat and its stability on retail display in order to develop strategies to reduce meat discolouration. This thesis evaluates different phenotypic and genotypic factors influencing lamb meat colour in Australia. Bloomed colour (L^*, a^*, b^*, hue angle and chroma) was measured in the loin muscle of over 8000 mixed breed lambs of known genetics produced at 8 sites across Australia over 5 years as part of the Sheep CRC's INF experiment. The colour stability of the loin muscle was evaluated in over 4000 of these lambs using spectrophotometric measures of meat redness (R630/R580) taken over a 3 day simulated retail display.

The first experiment of this thesis examines the influence of production factors and muscle traits on the bloomed colour of lamb loin. Over 40% of the 8165 loin samples

were too dark for consumer acceptance, suggesting that dark meat is a substantial problem in the Australian lamb meat industry. Production factors such as lamb slaughter group, production site and year of production had substantial effects on bloomed colour, though these effects could not be attributed to changes in muscle traits such as myoglobin or pH_{24}. Further investigation is required to better understand how production factors influence bloomed meat colour. Of the muscle traits analysed, changes in pH at 24 hours (pH_{24}) had the greatest effect on meat *a**, while myoglobin had the greatest effect on meat *L**. Increasing lamb age from 140 to 400 days reduced meat *L** due to increased myoglobin concentration. These results suggest that industry focus needs to shift to consideration of myoglobin concentration as well as meat pH in order to improve the bloomed colour of lamb meat, particularly meat lightness.

The second experiment examines the influence of selection for IMF and lean meat yield on bloomed colour. Increasing IMF from 2 to 8% and shortloin fat weight from 100-500g were positively associated with meat *L**, *a**, *b**, hue angle and chroma. Shortloin muscle weight was negatively associated with these colour parameters, though could largely be accounted for by correlated changes in IMF. The effect of sire breeding values for lamb weight, shortloin muscle depth and fat depth on loin *L**, *a**, *b**, hue angle and chroma were small and varied between lamb sire type, dam breed and sex. Thus selection for increased lean meat yield in lambs will have neutral or positive effects on meat colour, while selection for increased IMF will increase the *L**, *a**, chroma and thereby the consumer appeal of bloomed lamb meat.

The third experiment examines the influence of muscle weight and oxidative capacity on the colour of lamb loin following 72 hours of simulated retail display. Production factors such as slaughter group and site of production had the greatest magnitude effects on meat R630/R580 (redness) after 3 days of display. Increasing loin ICDH activity,

reflecting muscle oxidative capacity, reduced R630/R580 at the end of display. Selection for high sire breeding values for shortloin muscle depth increased R630/R580, likely due to an associated reduction in muscle oxidative capacity. Lamb carcass weight also increased R630/R580. Genotypic factors influencing lamb size and growth rate such as sire type and dam breed further support that increased growth rate improves meat colour. These findings suggest that breeding for increased growth rate and muscle weight could improve the colour stability of overwrapped lamb on retail display.

The fourth experiment examines the ability to predict the rate of lamb meat browning on display using measures of bloomed colour and information of animal factors that influence colour stability. Simple and partial correlation coefficients between initial boomed R630/R580 and subsequent R630/R580 measures over display were ≤ 0.4, despite incorporation of carcass traits including pH_{24}, lamb age and IMF. Therefore, bloomed colour at the start of display cannot provide a useful prediction of subsequent meat browning. Correlations between 24, 48 and 72 hr measures of R630/R580 were high (> 0.8), suggesting that colour measured from 24 hours of display can provide an accurate prediction of subsequent meat colour. While predicting the rate of meat browning would allow retailers to maximise the display time of lamb, meat colour measured at 24 hrs of display is unlikely to be practical in a retail setting. The predictive ability of 24 hr colour may therefore be limited to use in the development of a breeding value for retail colour.

The final experiment examines the use of dietary vitamin E supplementation to improve the colour stability of long-stored lamb meat, particularly meat with high IMF, pH_{24} and ICDH activity. The capacity of this antioxidant to improve the colour stability of lamb meat was expected to increase in long-stored meat with high IMF, pH_{24} and ICDH activity due to the anticipated increased oxidative load in this meat. Vitamin E supplementation at 275 mg/kg of feed for 8 weeks prior to slaughter increased R630/R580 throughout the

display of lamb loin following 5, 35 and 70 days of storage. However contrary to expectations, vitamin E supplementation had a similar magnitude impact on meat colour stability regardless of storage time prior to the display. Vitamin E did mitigate the negative effect of high IMF on short-stored meat colour, however high IMF did not impact the colour stability of medium or long-stored meat. These results demonstrate that while vitamin E supplementation will improve the colour stability of lamb meat following short, medium or long storage, its greatest impact is on the display colour of short-stored lamb with high IMF content.

Acknowledgments

Undertaking this thesis has been the most challenging and rewarding experience. I would certainly never have made it this far without the endless support of my three excellent supervisors - Associate Professor Graham Gardner, Prof Dave Pethick and Dr Robin Jacob. I am extremely grateful to these three, who have each contributed their unique perspectives and invaluable research expertise to this thesis. They have taught me an enormous amount, provided me with amazing opportunities and experiences, and we have become great friends in the process. I could not wish for better mentors.

This research would not have been possible without the financial and practical support of the Sheep Cooperative Research Centre (CRC). I met the Sheep CRC board on several occasions and always felt encouraged and supported in my research. I was proud to represent the Sheep CRC at the national CRC conference in 2014, and greatly valued the annual postgraduate student conference that was supported by the Sheep CRC. These conferences provided individual feedback on our research and presentation skills from industry experts and allowed us to meet other students undertaking research in the livestock industries. Thanks to my team at Murdoch and opportunities to meet colleagues across Australia and internationally, I never felt isolated in undertaking this PhD.

Murdoch University has provided welcome support and it has been great to return to the school where I completely my veterinary degree and get to know a whole new aspect to the university. I enjoyed being heavily involved in a lot of undergraduate teaching at Murdoch, which helped me get to know people in the school and broaden my skills in livestock research.

I feel very fortunate to have been part of such an excellent team at Murdoch. Essentially, morning coffees got me through this PhD! I thoroughly enjoyed running experimental work as part of such a large motivated team, and loved helping out with experimental work of colleagues in other areas of the animal and veterinary sciences. I was very fortunate to have been introduced to the team at Murdoch by Khama, following some brainstorming on career paths one quiet Saturday afternoon at Baldivis. I met with Graham the next week and as things tend to go with Graham- I was swept up in his enthusiasm and never looked back.

Thanks to everyone in our team for their assistance and support in my research. To Peter for rescuing me from my cubicle, helping me find my place in this team, and for infecting me with a passion for and sense of pride in Australian lamb and beef production. Big thanks to my office mates over the years, particularly Cam and Sarah-John, with whom some of my best memories were made. Big thanks to Fiona and Khama for showing me the way through the first couple of PhD years, to Sarah and Rachel for joining the team and becoming great friends, to Di and Kate for being convinced to come back to university with me! Thanks to Willo for his statistical skills and to Mal, Ken, Janice, Rini and Di for being the backbone of our team, keeping the work of the whole team running full steam ahead. Thanks to the lecturers around the traps- Dave Miller, Anne Barnes, John Pluske – who were always there for a word of support, a laugh, a coffee or a beer. Much appreciated.

I am grateful to my family for being there. To my parents for the constant support whilst hassling me to stop having fun and write my thesis. My lamb connections and newfound carvery skills undoubtedly raised me in the esteem of my siblings. Thanks to my friends and to Jason for putting up with me through it all. You're the best.

Publications

Journal publications:

Calnan, H.C., Jacob, R.H., Pethick, D.W. and Gardner, G.E. (2014). Factors affecting the colour of lamb meat from the *longissimus* muscle during display: The influence of muscle weight and muscle oxidative capacity. *Meat Science*. 96: 1049-1057.

Calnan, H.C., Jacob, R.H., Pethick, D.W. and Gardner, G.E. (2016). Production factors influence fresh lamb *longissimus* colour more than muscle traits such as myoglobin concentration and pH. *Meat Science*. 119: 41-50.

Calnan, H.C., Jacob, R.H., Pethick, D.W. and Gardner, G.E. (2016). Selection for IMF and lean meat yield will improve the bloomed colour of Australian lamb loin meat. *Meat Science*. 131: 187-195.

Journal publications to be submitted:

Calnan, H.C., Jacob, R.H., Pethick, D.W. and Gardner, G.E. Initial bloom colour is a poor predictor of colour stability in lamb *longissimus* meat.

Calnan, H.C., Jacob, R.H., Pethick, D.W. and Gardner, G.E. Dietary vitamin E supplementation improves the colour stability of lamb loin meat following up to 70 days of chilled storage.

Conference proceedings:

Calnan, H.C., Jacob, R.H., Pethick, D. W. and Gardner, G. E. (2017). Selection for increased IMF and lean meat yield can improve the bloomed colour of lamb meat.

Accepted abstract for the 63rd International Congress of Meat Science and Technology, Dublin, Ireland 2017.

Calnan, H.C., Jacob, R.H., Pethick, D. W. and Gardner, G. E. (2016) Retail lamb browning is poorly predicted using meat pH_{24} and colour measured at the start of display. Accepted abstract for the 62st International Congress of Meat Science and Technology, Bangkok, Thailand 2016.

Calnan, H.C., Jacob, R.H., Pethick, D. W. and Gardner, G. E. (2015) pH_{24} of lamb drives fresh meat redness more than myoglobin or iron content. Accepted abstract for the 66th Annual Meeting of the European Federation of Animal Production Warsaw, 2015. Book of Abstracts No.21 (Wageningen Academic Publishers).

Calnan, H.C., Jacob, R.H., Pethick, D. W. and Gardner, G. E. (2015) Vitamin E supplementation improves retail colour less in long aged lamb meat. Accepted abstract for the 61st International Congress of Meat Science and Technology, Clermont Ferrand, France 2015.

Calnan, H.C., Jacob, R.H., Pethick, D. W. and Gardner, G. E. (2014) Sire selection for muscling improves the lightness and redness of lamb meat. Accepted abstract for the 65th Annual Meeting of the European Federation of Animal Production, Copenhagen, Denmark 2014. Book of Abstracts No.20, p 229. (Wageningen Academic Publishers).

Calnan, H.C., Jacob, R.H., Pethick, D. W. and Gardner, G. E. (2014) Using Vitamin E to improve colour stability is less effective in long aged lamb meat. Proceedings of the Australian Society for Animal Production 30, 292, Canberra, Australia.

Calnan, H.C., Jacob, R.H., Pethick, D. W. and Gardner, G. E. (2014) Vitamin E did not improve retail colour in high IMF long aged lamb meat. In ‘Proceedings of Sheep CRC Postgraduate Student Conference’. Manly, New South Wales.

Calnan, H.C., Jacob, R.H., Pethick, D. W. and Gardner, G. E. (2013) Reducing the pH of lamb carcasses will improve retail meat colour. Proceedings of the 64th Annual Meeting of the European Federation of Animal Production Nantes, France. Book of Abstracts No.19, p 110. (Wageningen Academic Publishers).

*Calnan, H.C., Jacob, R.H., Pethick, D. W. and Gardner, G. E. (2013) Increased muscularity and reduced intramuscular fat of lambs will increase meat redness on retail display. Proceedings of the 59th International Congress of Meat Science and Technology, Izmir, S5-65. (Ege University, Food Engineering Development).

Calnan, H.C., Jacob, R.H., Pethick, D. W. and Gardner, G. E. (2013) Merino sires and high 24 hour pH reduce lamb meat colour stability. In 'Proceedings of Sheep CRC Postgraduate Student Conference'. Coffs Harbour, New South Wales.

Calnan, H.C., Jacob, R.H., Pethick, D. W. and Gardner, G. E. (2012) IMF and muscle aerobicity reduce colour stability. In 'Proceedings of Lambex 2012'. Bendigo, Victoria. Proceedings p 38.

Calnan, H.C., Jacob, R.H., Pethick, D. W. and Gardner, G. E. (2012) IMF and muscle oxidative capacity reduce colour stability. In 'Proceedings of Sheep CRC Postgraduate Student Conference'. Coffs Harbour, New South Wales.

*Presentation of this paper at the 59th International Congress of Meat Science and Technology in Izmir, Turkey was awarded the 2013 International Meat Secretariate Prize.

Abbreviations

*a**	Redness
AEC	Animal Ethics Committee
AMSA	American Meat Science Association
ASBV	Australian sheep breeding value
ATP	Adenosine triphosphate
*b**	Yellowness
CIE	Commission internationale de l'eclairage
CRC	Cooperative Research Centre
HCWT	Hot carcass weight
ICDH	Isocitrate dehydrogenase
IMF	Intramuscular fat
INF	Information nucleus flock
*L**	Lightness
MAP	Modified atmosphere packaging
MRA	Metmyoglobin reducing activity
MSA	Meat Standards Australia
MUFA	Mono-unsaturated fatty acids
NADH	Nicotinamide adenine dinucleotide
PEMD	Post-weaning eye muscle depth (ASBV)
PFAT	Post-weaning fat depth (ASBV)
pH_{24}	Meat pH measured at 24 hours *post-mortem*
pHu	Ultimate meat pH
PUFA	Poly-unsaturated fatty acids

PWT	Post-weaning weight (ASBV)
ROS	Reactive oxidant species
R630/R580	Reflectance at 630nm / reflectance at 580 nm

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Chapter 1. Introduction

There are a large number of factors that determine the quality of lamb meat and thus the willingness of consumers to purchase the product (Pethick 2006). Meat colour may be considered the most important factor given it largely determines the visual appeal of lamb meat on retail display. Consumers associate a bright red colour with meat quality and freshness, discoloured meat deterring their purchase. Retailers are therefore forced to discount or downgrade discoloured meat, resulting in substantial economic losses for the industry.

Meat colour is determined by myoglobin pigments (Fox Jr 1966). Myoglobin commonly exist in three forms in meat; deoxymyoglobin, oxymyoglobin and metmyoglobin. These pigments selectively absorb and reflect different wavelengths of visible light, giving each a unique colour – purple, red and brown respectively (Hunt 1980). Myoglobin changes forms by various oxygenation, oxidation and reduction processes, with the relative concentrations of myoglobin redox forms in meat determining its colour. Following exsanguination of an animal myoglobin exists predominantly in the unoxygenated form deoxymyoglobin, giving meat a purple colour. When meat is sliced and thereby exposed to oxygen, oxygen diffuses into the meat surface and binds with myoglobin to form oxymyoglobin, a process known as blooming that gives meat its bright red colour. With time following blooming, low oxygen concentrations in meat favour the auto-oxidation of myoglobin into metmyoglobin, the brown pigment responsible for meat browning. The colour stability of meat refers to the rate that meat browns following blooming, where meat with high colour stability browns at a slower rate than meat with low colour stability.

Successful retailing of lamb meat relies on bloomed colour and its stability on retail display. The most common problem associated with meat blooming is a dark meat colour. Dark meat is recognised in beef more than lamb due to the comprehensive carcass grading system for beef, Meat Standards Australia (MSA). Jose et al. (2015) estimated that the downgrading of dark beef under the MSA system cost the industry between $19 and $56 million (AUD) in 2014. The poor colour stability of lamb meat is also an important limitation to retailing lamb meat. Lamb meat in Australia is commonly overwrapped with oxygen-permeable clingfilm for retail display. Under these packaging conditions lamb meat maintains an acceptable red colour for only 2 to 3 days on display due to poor colour stability (Khliji, Van de Ven et al. 2010, Jacob, D'Antuono et al. 2014), compared to beef that remains red for over a week under the same conditions. Meat browning represents a substantial financial burden on red meat industries. Williams et al. (1992) estimated that increasing the shelf life of beef products by 1-2 days would save the US meat industry $175 to $1 billion (USD) annually. No work has quantified the cost of meat discolouration to the Australian lamb meat industry, however meat discolouration is widely recognised by industry to be an important limitation to the efficiency and profitability of the lamb meat supply chain. Better understanding of factors contributing to discolouration in lamb meat is needed for the industry to develop strategies in animal production and meat processing to improve meat colour.

Meat colour is influenced by a multitude of factors, directly and indirectly. Oxygen penetration into meat, binding with myoglobin and consumption via *post-mortem* metabolism directly influence bloomed meat colour and its stability on retail display. Carcass and muscle traits such as muscle oxidative capacity and pH influence these reactions and thus meat colour. In turn, a number of processing and production factors such as animal age and nutrition influence these carcass and muscle traits and thereby

indirectly influence meat colour. Due to the complexity and interrelatedness of the factors influencing bloomed meat colour and its stability on retail display, the influence of many phenotypic and genotypic on lamb meat colour are incompletely understood.

The INF experiment developed by the Australian CRC for Sheep Industry Innovation provides a unique opportunity to examine the impact of a range of genetic, production, processing, carcass and muscle factors on lamb meat colour. In this experiment the bloomed colour and its stability over a 3 day simulated retail display were measured in the *m. longissimus thoracis et lumborum* (loin muscle) of thousands of lambs produced at 8 sites across Australia over a 5 year period. Genetic factors such as sire type and breeding values, production factors such as site of production, year of birth, sex and kill group and phenotypic carcass traits such as hot carcass weight (HCWT), loin myoglobin concentration and pH were captured for each animal. A colorimeter was used to measure *L**, *a** and *b** of bloomed loin muscle at 24 hours *post-mortem* and to calculate hue angle and chroma. The loin muscle was stored chilled for 5 days before a fresh surface was sliced, bloomed and overwrapped for spectrophotometric measurement of meat redness (R630/R580) over a 3 day simulated retail display. An additional experiment examined the influence of dietary vitamin E supplementation on the R630/R580 of loin meat over retail display, particularly in lamb loin predisposed to browning and in meat stored chilled for up to 70 days prior to retail display. The data produced by these experiments provides the opportunity to learn more about phenotypic and genotypic influences on lamb meat colour in Australia and will assist in the development of industry strategies to reduce discolouration in lamb meat.

Chapter 2. Literature Review

2.1 The Australian lamb meat industry

Australia is the world's largest exporter of sheep meat and is the world's second largest producer of lamb and mutton (MLA 2016). The Australian sheep meat industry was estimated to have had an off-farm meat value (domestic expenditure and export value) of $4.83 billion in 2015-16 (MLA 2016). From a national flock of 75.5 million head, Australia produced 516,366 tonnes carcase weight of lamb and 196,040 tonnes of mutton in 2015-16 (MLA 2016). Over 90% of the mutton and 56% of the lamb produced in Australia were exported, making Australia the world's largest exporter of mutton and second largest exporter of lamb meat. Sheep meat is the fourth most commonly consumed meat in Australia following chicken, beef and pig meat (MLA 2016), over 80% of households purchased lamb in 2015-16 and Australians consuming an average of 9.5 kg of lamb each year (MLA 2016).

While Australians are some of the highest consumers of sheep meat in the world, Mongolians consume the most sheep meat per capita and China produces the most sheep meat (MLA 2016). Lamb meat is also widely consumed in Mediterranean countries, though its high price and consumption for special occasions means lamb meat is considered a luxury food. The consumption of lamb in Australia is also limited by its high cost relative to other meats. The highest quality lamb products retail for around $44 AUD per kilo currently. In Australia, lamb meat is typically sliced and displayed in butcher and supermarket cabinets for individual consumer sale. The majority of lamb meat is wrapped with oxygen permeable cling-film, though modified-atmosphere-packaging (MAP) and vacuum packaging of lamb is becoming increasingly common.

Consumers demand lamb meat that is lean, palatable and has good nutritional attributes, which drives their purchase and 'willingness to pay' decisions (Pethick 2006). The appearance of lamb meat on retail display is critical to consumer appeal and purchasing decisions.

2.2 The importance of meat colour to the lamb meat industry

Key attributes of fresh meat quality are nutritional value, consumer safety and consumer acceptability (Clydesdale and Ahmed 1978). Consumer acceptability involves many factors including the aroma, texture, colour, flavour, convenience and cultural relevance of the meat. Of the factors determining retail appeal, meat aroma and texture cannot be evaluated at retail without opening packages, thus consumers rely heavily on meat colour as an indicator of meat freshness and quality at the point of sale (Faustman and Cassens 1990). In determining visual appeal, meat colour may be considered the most important factor governing a consumer's decision to purchase sliced lamb meat (Mancini and Hunt 2005).

Consumers expect and demand that lamb meat is bright red in colour, often described as 'cherry' or 'brick' red. Consumers relate the red colour of meat to its freshness and thus any discolouration of lamb meat will deter a consumers' purchase. Discolouration of fresh red meat may be divided into two broad categories: a) the failure of meat to develop a bright red colour with blooming (oxygenation) and b) the gradual development of a brown surface colour following blooming.

2.2.1 Bloomed meat colour

Blooming refers to the change in meat colour that occurs after slicing exposes a meat surface to atmospheric oxygen. To an observer, the meat becomes redder and lighter in

colour during blooming as oxygen penetrates several millimetres into the meat surface and binds with myoglobin to form the red pigment oxymyoglobin (AMSA 2012). Beef and lamb meat must be allowed time following oxygen exposure to fully bloom and develop the premium bright red surface colour expected and demanded by consumers.

Failure of meat to bloom adequately results in meat that is less red and/or darker in colour. 'Dark cutting' in beef refers to dark meat colour resulting from a high ultimate pH (pHu). The effect of pH on bloom colour is twofold. Firstly oxygen consumption by mitochondria is greater when pH is high so the depth of oxygen penetration is reduced. Secondly the spacing of myofibrils is greater when pH is high so light absorption is increased. Together these effects cause the meat to be less red in colour and darker in colour accordingly. Quality aspects associated with high pHu include a dry texture, variable tenderness and greater susceptibility to bacterial spoilage (Borch, Kant-Muermans et al. 1996). Consumers may also perceive dark meat to be from older animals (Jeyamkondan, Jayas et al. 2000). Thus consumers of beef have come to associate dark red meat with poor palatability and avoid purchasing dark meat.

The Australian beef grading system known as Meat Standards Australia (MSA) includes standards for pHu and colour. A pHu of greater than 5.7 or an Ausmeat meat colour score greater than 3 is defined as dark cutting. Dark cutting has been estimated to cost the Australian beef industry up to $55 million AUD in the 2013/2014 financial year through downgrading of dark carcasses under MSA (Jose, McGilchrist et al. 2015). High pHu also occurs in lamb meat, however without the equivalent scope of carcass grading in the lamb meat industry, the incidence of dark cutting lamb carcasses and economic losses associated with this problem are difficult to estimate.

2.2.2 Colour stability

Following blooming, fresh red meats gradually develop a brown surface discolouration. For the purpose of this thesis, "browning" refers to the colour change in fresh meat caused by the oxidation of myoglobin pigments to form the brown pigment metmyoglobin. The deterioration of bloomed meat colour and development of surface browning is the major factor limiting the shelf life of fresh red meats, particularly lamb meat (Jeyamkondan, Jayas et al. 2000). Consumers reject brown meat as they associate browning with an absence of freshness, excess bacterial growth and incipient spoilage (Jeyamkondan, Jayas et al. 2000). However the perception that meat browning results from bacterial spoilage is most often incorrect. While bacterial spoilage can cause myoglobin oxidation, bacterial spoilage usually takes longer than myoglobin oxidation following oxygenation. While myoglobin oxidation in the absence of bacterial spoilage does not impact product safety or eating quality, consumers cannot necessarily distinguish the cause of meat browning and will continue to associate browning with reduced freshness and spoilage. Therefore meat browning will reduce the consumer appeal and retail value of lamb regardless of meat quality. Hence the shelf life of lamb meat under retail conditions is determined by browning rather than bacterial spoilage.

Retailers minimise the associated financial losses by heavily discounting browned meat, by trimming away browned portions of meat or by downgrading the meat to a lower valued product such as mince. These practices lead to large economic losses for the industry as a whole and to wastage of a valuable high quality protein source (Faustman and Cassens 1990). By limiting the shelf life of red meats after retail preparation, meat browning has also been the main challenge to centralising retail preparation (Jeyamkondan, Jayas et al. 2000).

Meat browning is also described as colour stability (AMSA 2012). Meat with high colour stability will remain red for a greater length of time on retail display. Lamb meat has lower colour stability than beef, meaning it browns at a faster rate following blooming (Atkinson and Follett 1973). Though the reasons for this remain unclear, higher oxygen consumption in lamb meat may be key (Atkinson and Follett 1973). For lamb meat overwrapped with oxygen-permeable film the bloomed meat colour will change from red to brown in one to seven days, depending on conditions (Moore and Young 1991). Anecdotal evidence suggests that Australian supermarket chains display overwrapped lamb meat for only 2 days before discounting the meat to ensure rapid sale and to prevent visible browning causing consumer rejection.

2.2.3 Financial costs of poor colour

Meat discolouration causes discounting, downgrading and waste of high quality meat and thereby results in substantial economic losses for red meat industries. These practices were estimated to cost the US beef industry more than $1 billion annually in 2000 (Smith 2000). Jose et al. (2015) estimated that downgrading of dark beef under the MSA grading system costs the industry $50 million AUD in 2015. Quantifying the cost of lamb discolouration to the Australian industry is difficult given a comprehensive carcass grading system such as MSA does not yet apply to this industry and as the cost of reduced shelf life is difficult to quantify. Williams et al. (1992) reported that the average loss of meat sales due to colour deterioration was 3.7% for the USA. If this rate was applied to the 516,366 tonnes of lamb and 196,040 tonnes of mutton produced in Australia in 2015-16, valued at 605 and 408c/kg respectively (MLA 2016), then an annual loss of over $145 million AUD would result.

2.3 Measuring Meat Colour

There is no single gold standard method for measuring meat colour. In reporting meat colour measures, substantial consideration needs to be given to the challenges inherent in communicating colour, including differences between instruments and their specifications, limitations of instrumental colour measurements and environmental factors that influence the perception of colour.

2.3.1 The perception of meat colour

A complex and synergistic interaction between the eye and the brain develops human colour perception (AMSA 2012). Instrumental measurement of colour provides a useful alternative to human surveys of meat colour (Khliji, Van de Ven et al. 2010). However communicating colour can be quite challenging, thus several tools have been developed to facilitate colour communication. The Munsell system was invented by the American artist A.H Munsell using colour chips to match to specimens. The commission Internationale de l'Eclairage (CIE) developed the tristimulus values XYZ (Figure 2-1) in 1931 and the CIE *L*a*b** colour space in 1976 (Figure 2-2). The CIE *L*a*b** system was developed as the XYZ colorimetric distances between individual colours did not correspond to perceived colour differences. For example the difference between green and greenish-yellow was relatively large while the distance between blue and red was relatively small, contrary to the perceived differences between these colours.

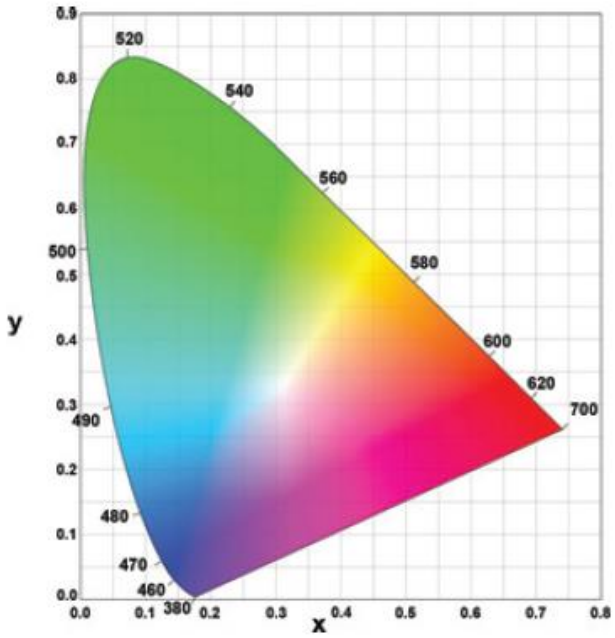

Figure 2-1 The Commission Internationale de l'Eclairage (CIE) 1931 colour space (AMSA 2012)

Perceived colour differences correspond to colorimetric distances in the CIE *L*a*b** colour space, where colour is expressed in a three dimensional space (Figure 2-2). The centre of the colour space is a neutral grey. Along the *a** axis, positive *a** values represent red, and negative *a** values represent green (scale from −60 for green to +60 for red). Along the Y axis, positive *b** values represents yellow and negative *b** values represents blue (scale −60 for blue to +60 for yellow). In the third dimension, *L** or lightness is represented numerically where 0 is black and 100 is white (Figure 2-2).

Perceptible colour has hue, lightness and saturation properties. Hue describes a shade of colour developed by specific wavelengths, lightness describes the total light reflected by all wavelengths, while saturation describes how vivid or dull a colour is. Hue and chroma (colour saturation) are calculated from *a** and *b** values in the *L*a*b** colour space; where hue = arctangent (b^*/a^*) and chroma = $(a^{*2} + b^{*2})^{1/2}$. Authors need to clarify whether CIE *L*a*b** values or CIE Lab values were used when reporting

colorimeter values in research, as the presence of the asterisks reflects slight mathematical differences in calculations.

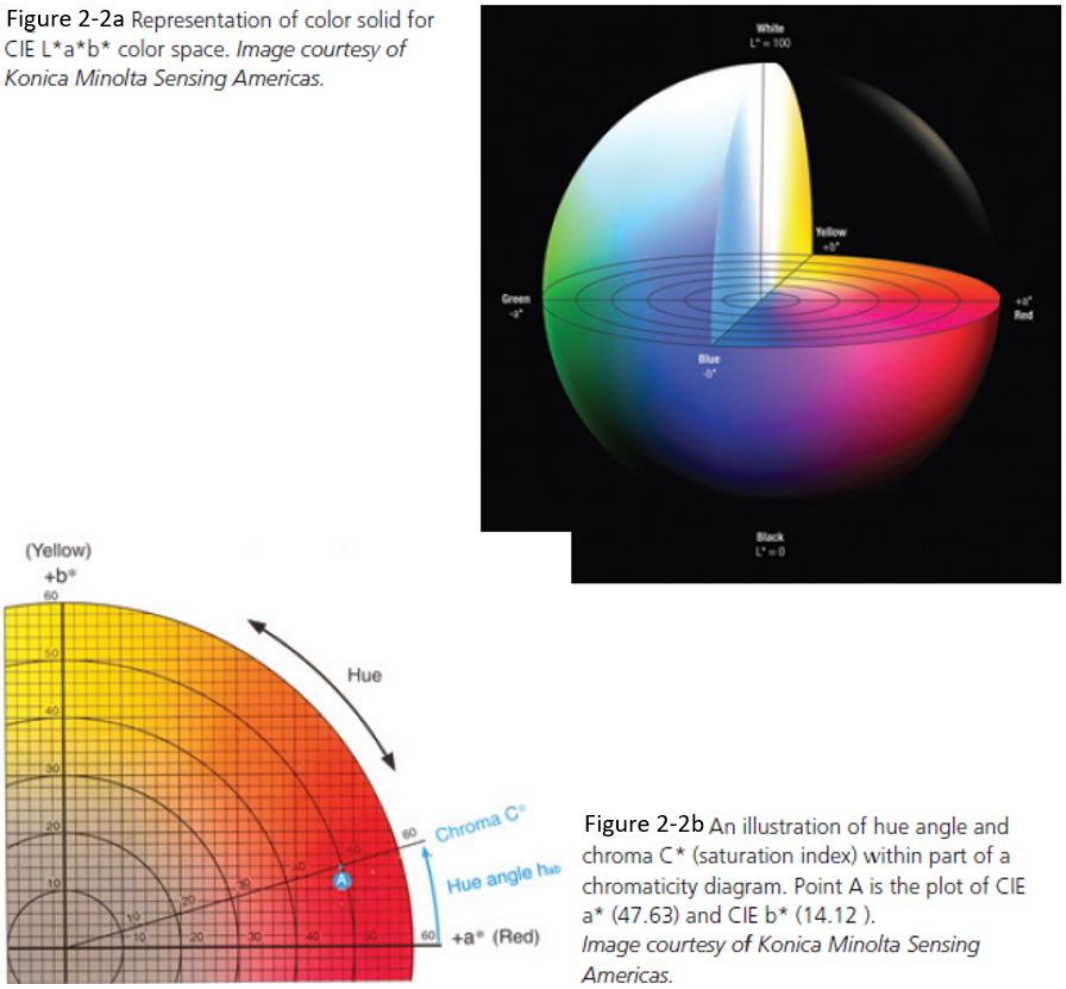

Figure 2-2a Representation of color solid for CIE L*a*b* color space. *Image courtesy of Konica Minolta Sensing Americas.*

Figure 2-2b An illustration of hue angle and chroma C* (saturation index) within part of a chromaticity diagram. Point A is the plot of CIE a* (47.63) and CIE b* (14.12). *Image courtesy of Konica Minolta Sensing Americas.*

Figure 2-2 Representations of the CIE *L*a*b** colour space (AMSA 2012)

2.3.2 Instrumental colour measurement

The two most advanced types of colour measurement instruments are colorimeters and spectrophotometers. Both instruments produce a light source with defined illuminant conditions, though measure the reflected light from a surface such as meat in different ways (AMSA 2012).

2.3.2.1 Colorimeters

Colorimetry rapidly measures colour with an inexpensive handheld device and has been used for many years to measure surface meat colour (MacDougall 1982). Colorimeters commonly use a set illuminant, CIE 10 degree observer and a tristimulus absorption filter to emulate the response of the human eye to light. Light reflected from a meat surface passes through red, green and blue filters to photo-detectors and is converted into XYZ or CIE *L*a*b** values (Figure 2-3).

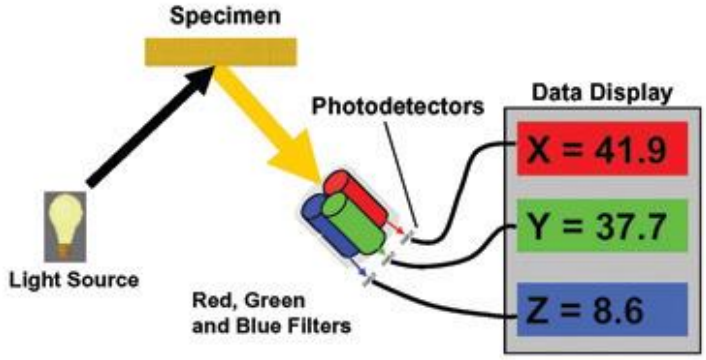

Figure 2-3 Colour measurement with a tristimulus colorimeter (AMSA 2012)

2.3.2.2 Spectrophotometers

Spectrophotometers supply spectral analyses in intervals of 1 to 10 nm and offer several illuminant/observer combinations for the calculation of tristimulus values. Spectrophotometers illuminate the sample and measure reflected light waves by scanning (via a monochromator) or reading simultaneously with a photo diode array (Figure 2-4). For meat colour research, the spectral analyses provided by spectrophotometers allows the proportions of different myoglobin forms to be inferred, which cannot be achieved using a colorimeter (Hunt 1980).

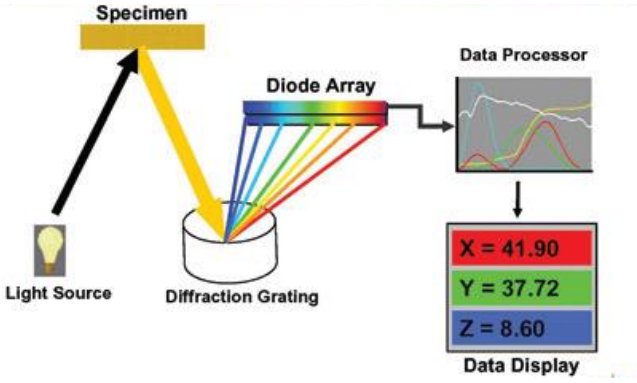

Figure 2-4 Spectrophotometric colour measurement (AMSA 2012)

2.3.2.3 *L**, *a**, *b**, hue and chroma in meat colour research

CIE *L*a*b** values describe the lightness (*L**), redness (*a**) and yellowness (*b**) of meat colour and permit the calculation of hue and chroma. In terms of meat colour, increased *L** is preferred by consumers due to their association of dark meat with quality defects (Khliji, Van de Ven et al. 2010). Increased *a** values are also sought by consumers due to demand for bright red lamb meat, however the importance of *b** values consumer preference for meat colour are unclear. Increasing *b** values do however correspond to higher values of chroma, which represents a more vivid colour and a lack of greyness (Miltenburg, Wensing et al. 1992), that is desirable to consumers.

In a study examining the relationship between consumer ranking and objective measures of lamb meat colour, Khliji et al. (2010) found that chromametric values of $a^* \geq 9.5$ and $L^* \geq 34$ were required for average consumer acceptance of loin meat colour. However, values of $a^* \geq 14.5$ and $L^* \geq 44$ are needed for 95% confidence that a randomly selected consumer deems the meat colour acceptable (Khliji, Van de Ven et al. 2010). Alternatively, Hopkins (1996) reported that *a** values > 19 and *L** values > 35 were required for consumers acceptance of lamb meat colour. The substantially different *a** threshold values reported by Khliji et al. (2010) and Hopkins (1996) is

likely a result of different machine specifications, particularly the aperture size selection given its impact on *a** values.

Data relating consumer acceptance of lamb colour to measures of *b**, chroma and hue is scarce. Thresholds for consumer acceptance of lamb meat colour in terms of *b**, chroma and hue are unknown, with desired values for these measures is presumed based on consumer preference for bright red meat- low *b**, high chroma and hue values close to 0º (red).

2.3.2.4 R630/R580 in meat colour research

Spectrophotometric measures of light reflectance at different wavelengths are useful to estimate different myoglobin forms present in a meat surface. Meat redness may be estimated using measures of light reflectance at 630nm ÷ reflectance at 580nm (R630/R580). This ratio represents the redness: brownness of a meat surface (AMSA 2012), as oxymyoglobin pigments are highly reflected at 630nm relative to metmyoglobin pigments, and vice versa at 580nm (Figure 2-5 Reflectance of deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb) at the wavelengths used to indicate redness in raw meat (R630/R680) (AMSA 2012).(AMSA 2012). This ratio is also referred to as redness or oxy/met. The ratio is not simply a measure of oxymyoglobin: metmyoglobin however, as deoxymyoglobin pigments are also highly reflected at 630 nm (AMSA 2012) (Figure 2-5).

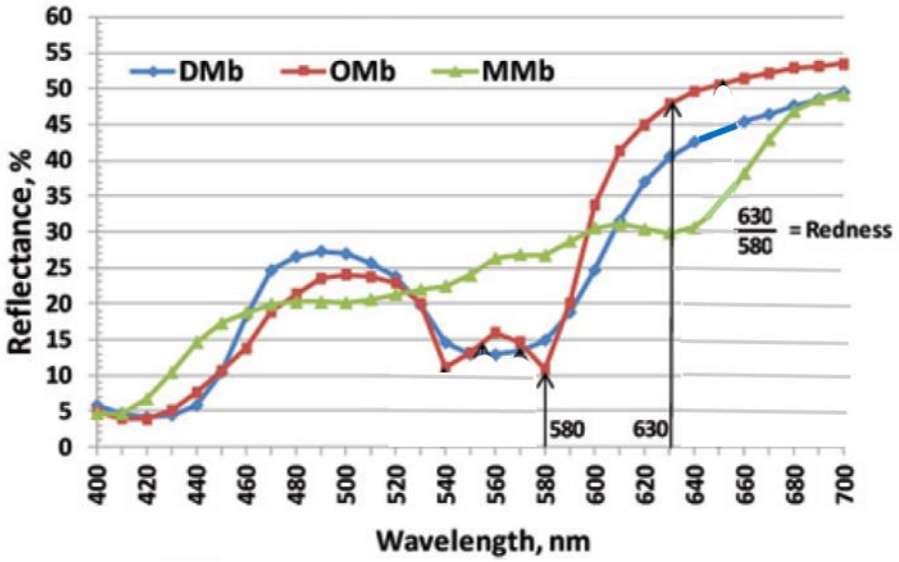

Figure 2-5 Reflectance of deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb) at the wavelengths used to indicate redness in raw meat (R630/R680) (AMSA 2012).

R630/R580 increases during blooming of red meat as oxymyoglobin is formed, before gradually decreasing with time as metmyoglobin accumulates beneath the meat surface. R630/R580 approaches a minimum value after 3 days of simulated retail display in overwrapped lamb meat (Jacob, D'Antuono et al. 2014). R630/R580 needs to exceed 3.3 units for the average consumer to deem lamb loin meat an acceptable red colour (Khliji, Van de Ven et al. 2010). However a R630/R580 of 6.3 is needed for 95% confidence that a randomly selected consumer would consider the meat to be an acceptable colour (Khliji, Van de Ven et al. 2010). The difference in these threshold R630/R580 values demonstrates the wide variation in individual consumer perception of red meat colour.

Khliji et al. (2010) reported that overwrapped lamb loin had an average R630/R580 of 6.0 after blooming which decreased over 3 days of simulated retail display to 3.3. The loin R630/R580 decreased below the threshold of consumer acceptability at 2.5 to 3 days of display (Khliji, Van de Ven et al. 2010). Morrissey et al. (2008) reported that an R630/R580 < 3.5 in topside was perceived by consumers to be more brown than red and therefore unacceptable, and that the relationship between consumer scores and

R630/R580 of the loin was relatively poor. However Khliji et al. (2010) surveyed a larger number of consumers than Morrissey et al. (2008). Thresholds of < 3 have also been reported for consumer acceptance of lamb meat R630/R580 (Jacob, D'Antuono et al. 2007), while Toohey and Hopkins (2006) reported that the acceptability threshold is < 3 and varies according to the meat cut. R630/R580 is influenced by aperture size (Hopkins, Kerr et al. 2008, Holman, Ponnampalam et al. 2015), thus different aperture size may have influenced the thresholds reported.

2.3.2.5 Instrument specifications and considerations

Instruments specifications including the illuminant, degree of observer, aperture size and standardisation procedure can vary. These factors interact with meat factors due mainly to the translucency of meat (Swatland 2012). As a result, substantial variation exists in *L**, *a** and *b** values reported in red meat studies. CIE *L**,*a**,*b** values should not be compared directly between studies without particular attention to the instrument and specifications used. The most commonly selected degrees of observer are 2º and 10º observers. The 10º observer is most commonly used for meat colour measurement and is recommended by the American Meat Science Association (AMSA) because it captures a larger portion of the sample (AMSA 2012). Illuminant A places more emphasis on the proportion of red wavelengths and is recommended for measuring meat colour. Values for *a** can vary by 5 to 25 units for the same sample depending upon the illuminant used. *a** values for freshly bloomed beef meat are typically 30 to 40+ for illuminant A, and only 20 to 30+ for Illuminant C or D65.

Measurement of meat colour also depends on the aperture size (AMSA 2012). Aperture size selection and reporting in meat colour research is critical to interpreting and comparing data (Kropf 1993, Tapp, Yancey et al. 2011, Holman, Ponnampalam et al. 2015). As aperture size decreases, the percentage reflectance, particularly of red

wavelengths between 600 and 700 nm, also decreases (Yancey and Kropf 2008). R630/R580 will therefore reduce with reducing aperture sizes. Hopkins et al. (2008) found that R630/R580 reduced when measured using hunterlab spectrophotometer with a 5mm aperture compared to a 25mm aperture. CIE *L*a*b** values also decrease with decreasing aperture size, with the largest differences seen in *a** values. Aperture selection should be based on the size of the meat sample- the largest size aperture that allows multiple measurements of the same sample is recommended (AMSA 2012, Holman, Ponnampalam et al. 2015). Holman et al. (2015) found that the effect of aperture size on lamb meat colour measurements varied with display time and muscle type.

The size, thickness, cut and uniformity of meat samples is also important in meat colour measurement. Differences in sample size can affect perceived colour as it affects the amount of reflected light. Samples are recommended to be at least 12 to 15 mm in thickness to ensure sufficient absorption of non-reflected light (AMSA 2012). The viewing angle and incident angle of light from the illuminant source will also impact colour perception (AMSA 2012). For example, the incident angle of light to the observer can determine whether iridescence is visible on a meat surface. The background of samples should be standardised and the size, colour and structural uniformity of a sample surface should be considered. Specific areas may be severely discoloured, contain intramuscular fat (IMF) or seams of connective tissue that disrupt the sample uniformity. Maximising the uniformity of samples and averaging multiple instrumental scans for analysis are recommended (AMSA 2012).

2.4 Mechanisms of meat colour

Pigments are materials that change the colour of reflected or transmitted light as the result of wavelength-selective absorption. Pigments in meat therefore determine meat colour. Myoglobin is the primary pigment responsible for red meat colour, though other heme protein pigments such as haemoglobin and cytochrome C may also play a role (Bekhit and Faustman 2005). Haemoglobin is the primary pigment of blood and has been reported to make up 6 - 16% of all pigments in fresh meat (Bekhit and Faustman 2005). Though the majority of haemoglobin is removed from muscles when an animal is exsanguinated, residual blood may be trapped in arteries and veins within large skeletal muscles (Suman, Hunt et al. 2014). Haemoglobin and cytochrome pigments are more important determinants of colour in poultry, fish and game meats than in beef or lamb meat (Suman and Joseph 2013).

2.4.1 Myoglobin

Myoglobin is water soluble and is present in the sarcoplasm of skeletal and cardiac muscle cells of almost all mammals (AMSA 2012).

2.4.1.1 Myoglobin structure

Myoglobin is a small protein with a molecular weight of approximately 17,000 da and a simple structure consisting of 8 α helices made up of 140 to 160 amino acid residues and no cysteines (Antonini 1965, Renerre 1990). Within the hydrophobic core of myoglobin is a heme prosthetic group that consists of a centrally located iron atom with six coordination sites. Four of these coordination sites are in the plane of and bound to the nitrogen atoms of four flat porphyrin rings. The remaining two coordination sites lie perpendicular to this structure. One of these perpendicular coordination sites is connected to the external nitrogen atom of the proximal histidine imidazole of the

globin protein molecule (Giddings and Solberg 1977). The sixth coordination site of myoglobin is open and available for a variety of ligands to bind and share a pair of electrons (Govindarajan, Hultin et al. 1977). The type of molecule attached to this sixth binding site and the oxidation state of the iron atom (ferrous or ferric) determines the properties of myoglobin, including its colour and reactivity (Renerre 2000).

2.4.1.2 Physiological function of myoglobin

Myoglobin is the primary pigment for oxygen transport within both cardiac and skeletal muscle cells (Ordway and Garry 2004). Wittenberg and Wittenberg (2003) found that oxygen within a muscle cell was predominantly bound to myoglobin; at a ratio of approximately 30:1 of bound: free oxygen. Myoglobin is produced in response to the demand of mitochondria for oxygen (Millikan 1939), as the ability of myoglobin to reversibly bind oxygen allows it to transport, store and supply oxygen for mitochondrial metabolism. Myoglobin thereby enables physiological muscle contraction (Millikan 1939, Wittenberg 1970). While myoglobin's primary role is in oxygen storage and delivery, myoglobin has also been proposed to have regulatory (Wittenberg and Wittenberg 2003) and protective functions within muscle fibres (Brunori 2001). In meat, the structure and redox forms of myoglobin determine meat colour.

Myoglobin differs between animal species (AMSA 2012), sheep myoglobin being most similar to goat and cattle myoglobin (Suman and Joseph 2013).

Table 2-1 Percentage sequence similarities between red meat and poultry myoglobins (Suman and Joseph 2013)

Species	Beef	Buffalo	Sheep	Goat	Pig	Chicken
Beef	100					
Buffalo	98.0	100				
Sheep	98.7	96.7	100			
Goat	97.4	95.4	98.7	100		
Pig	88.2	86.9	89.5	88.2	100	
Chicken	72.5	71.2	72.5	71.9	76.5	100

2.4.1.3 Myoglobin reduction oxidation (redox) forms

There are three main chemical forms of myoglobin that determine meat colour; deoxymyoglobin, oxymyoglobin and metmyoglobin (AMSA 2012). These redox forms of myoglobin differ based on the presence of oxygen and the redox state of the iron atom within the heme moiety.

- *Deoxymyoglobin* – myoglobin containing ferrous iron (Fe^{2+}) with a vacant sixth coordination site. Deoxymyoglobin has a purple colour that results in the dark purplish-red colour typical of the inside of fresh meat or of raw meat in vacuum packaging.

- *Oxymyoglobin* –formed when diatomic oxygen attaches to the sixth coordination site of ferrous iron (Fe^{2+}) in deoxymyoglobin. The bound diatomic oxygen interacts with the distal histadine-64 producing a more compact protein structure that is more stable than deoxymyoglobin. Oxymyoglobin is a red pigment and is responsible for the bright red colour typical of fresh red meats such as lamb (AMSA 2012).

- *Metmyoglobin* – the oxidised form of myoglobin containing ferric iron (Fe^{3+}). Water is bound to the sixth coordination site (Takano 1977) in metmyoglobin, preventing oxygen from binding (Hood and Riordan 1973) and making metmyoglobin physiologically inactive (Faustman and Cassens 1990) and more stable than deoxymyoglobin or oxymyoglobin. Metmyoglobin forms readily at low oxygen concentrations (< 7 mmHg or about 1 – 2 % oxygen) and has a dull tan to brown colour responsible for the visible surface browning of red meat (AMSA 2012).

Meat colour can also be influences by myoglobin binding with carbon monoxide (CO) or nitrous oxide (NO). CO influences fresh meat colour as it binds with myoglobin in the absence of oxygen to form carboxymyoglobin, which gives meat a stable bright red/pink colour. However, the use of CO is not permitted in Australia. NO alters the colour of cured meats and does not influence the colour of fresh lamb meat on display.

2.4.2 Myoglobin redox & oxygen binding dynamics

The dynamic reactions between deoxymyoglobin, oxymyoglobin and metmyoglobin determine the colour of lamb meat at a particular point in time (ASMA 2012; Figure 2-6).

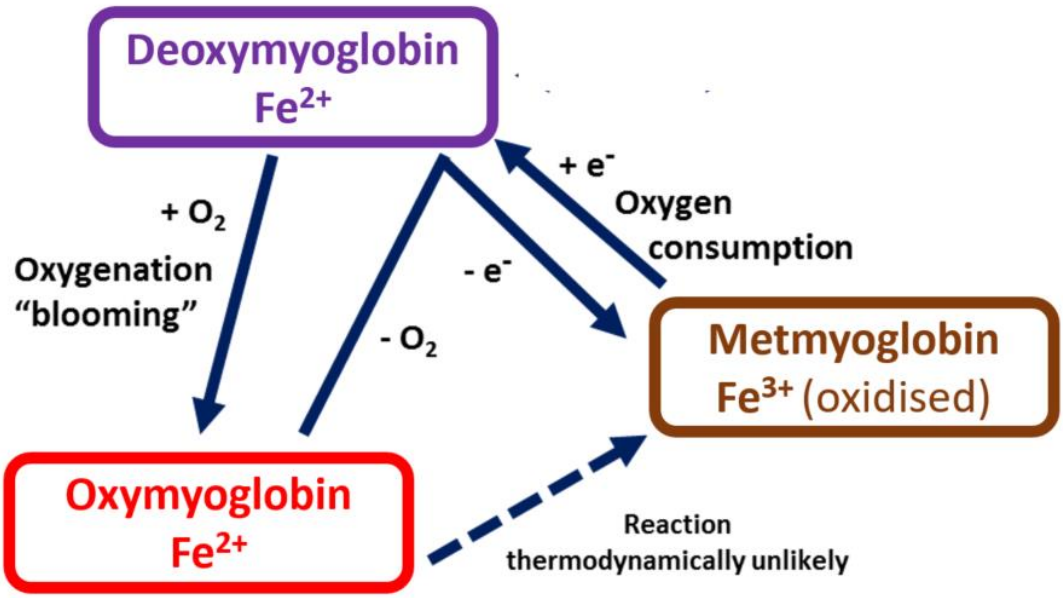

Figure 2-6 The reactions dynamic between the three main chemical forms of myoglobin important in determining red meat colour (AMSA, 2012).

2.4.2.1 Myoglobin oxygenation (meat blooming)

In live animals the majority of myoglobin is present in the oxygenated form (oxymyoglobin) as it functions to store and deliver oxygen to mitochondria, giving muscle tissue a bright red colour. However, with exsanguination of an animal post-slaughter, oxygen delivery to muscles is effectively terminated. Oxidative metabolism continues *post-mortem* and rapidly utilises oxygen remaining in muscle tissues, creating an anoxic environment where purple deoxymyoglobin predominates. The meat within a carcass will therefore appear dark red to purple in colour, as commonly observed when meat is first cut or is packaged in a vacuum devoid of oxygen.

Oxygen diffuses into a meat surface with blooming, binding to deoxymyoglobin to rapidly form oxymyoglobin (Faustman and Cassens 1990), giving the meat a bright red colour (Fox Jr 1966). The conversion of deoxymyoglobin to oxymyoglobin is a reversible reaction (Figure 2-6). A number of factors influence the depth of oxygen

penetration (or ‘bloom depth’) and thereby the bloomed colour of meat and its subsequent rate of browning. Myoglobin remains deoxygenated beneath the bloom depth, meaning that purple deoxymyoglobin lies beneath the red oxymyoglobin layer of a bloomed meat surface. Meat colour instruments measure light reflectance following penetration of wavelengths into a meat surface, thus may be influenced by deoxymyoglobin pigments in addition to surface oxymyoglobin pigments depending on the bloom depth of the meat and light penetration of the instrument. Increased bloom depth increases the proportion of oxymyoglobin: deoxymyoglobin in a meat surface and thus the perceived redness and lightness of the meat.

A range of physical and biochemical factors influence bloom depth. Oxygen penetrates further into meat that has a low pH, reduced enzyme activity and a more open structure (Cornforth and Egbert 1985). Increased oxygen consumption reduces the depth of oxygen penetration (Monin and Ouali 1992). The concentration of oxygen exposed to a meat surface strongly influences bloom depth. Exposure to atmospheric oxygen (21%) results in a typical bloom depth of 3-4 mm deep in red meat (Winstanley 1979). Exposure to high oxygen concentrations in modified atmosphere packaging (MAP) increases bloom depth and thus improves bloomed colour and its stability on retail display.

2.4.2.2 Myoglobin oxidation

Differences in the rate of myoglobin oxidation primarily determines the rate of meat browning (O'Keeffe and Hood 1982). The oxidation of myoglobin (Fe^{2+}) into metmyoglobin (Fe^{3+}) is often referred to as ‘auto-oxidation’ as it is a spontaneous and non-enzymatic reaction that involves free oxygen (Giddings and Solberg 1977). The reaction is highly dependent on oxygen concentrations (Brooks 1935) and is most rapid at low oxygen partial pressures of 1 - 1.4 mmHg (Figure 2-7) (George and Stratmann

1952). Metmyoglobin forms 10-20 times faster at 1-2% oxygen than at 21% atmospheric oxygen (Giddings and Hultin 1974).

Oxygen concentration are low at the depth of its penetration with blooming, therefore bloom depth is associated with colour stability. Metmyoglobin first forms at the bottom of the bloom layer, where low oxygen concentrations favour myoglobin oxidation. With time following blooming, mitochondrial activity depletes oxygen concentrations in meat and produces reactive oxidant species, which both trigger myoglobin oxidation and the accumulation of metmyoglobin. Metmyoglobin therefore begins forming at the depth of blooming and accumulates towards the meat surface with time following blooming, eventually causing visible meat browning. Reduced bloom depth will therefore cause metmyoglobin to form closer to the meat surface and more rapidly result in visible meat browning (Winstanley 1979).

As myoglobin oxidation will not occur in the absence of oxygen or oxymyoglobin, most studies investigate metmyoglobin development from oxymyoglobin. However, the direct conversion of oxymyoglobin to metmyoglobin is considered thermodynamically unlikely (AMSA 2012) (Figure 2-6). Deoxymyoglobin is less stable than oxymyoglobin (Giddings and Solberg 1977) and is thereby the most likely origin of metmyoglobin (Yusa and Shikama 1987). A dynamic dissociation equilibrium exists in meat where oxymyoglobin is continually converted to deoxymyoglobin plus oxygen and vice versa (AMSA 2012), therefore oxymyoglobin is likely deoxygenated into deoxymyoglobin before being rapidly oxidised to metmyoglobin.

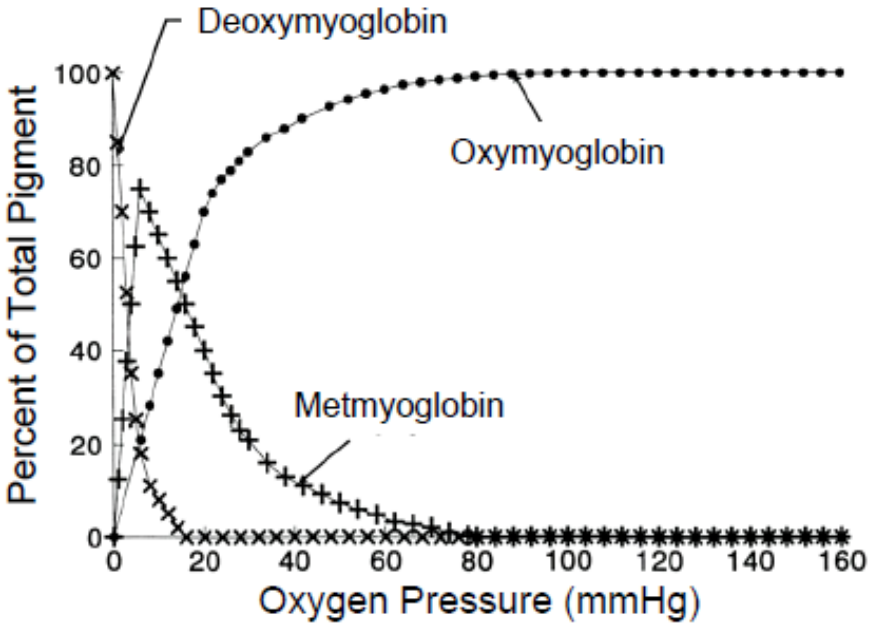

Figure 2-7 The effect of oxygen partial pressure on the relative concentrations of oxymyoglobin, deoxymyoglobin and metmyoglobin. Adapted from Forrest, Aberle, Hedrick, Judge & Merkel (1975).

Reactive oxygen species (ROS) present in meat trigger myoglobin oxidation (Faustman, Sun et al. 2010). ROS are intracellular chemically reactive oxygen-containing species formed as a natural by-product of oxygen metabolism that play important roles in cell signalling and homeostasis. However, in times of oxidative stress ROS react with and damage various intracellular biomolecules. ROS are therefore most active in systems such as meat that are under oxidative stress. Free radicals are ROS that are overly active due to the presence of an unpaired electron, making the radical unstable and highly reactive. Free radicals will often react with one another, generating further oxidative species through a series of chain reactions. Free radicals readily bind and damage biomolecules such as myoglobin.

Lamb meat browns at a much higher rate than beef, despite the 98.7% sequence similarity between sheep and beef myoglobin (Table 2-1). However the cause of the increased rate of myoglobin oxidation in lamb meat remains unclear.

2.4.2.3 Metmyoglobin reduction

Metmyoglobin does not accumulate in muscle tissue under physiological conditions, providing strong evidence that an active system operates in muscle to reduce metmyoglobin (Dean and Ball 1960). A number of different enzymatic systems and non-enzymatic systems have since been reported to reduce metmyoglobin, collectively referred to as 'metmyoglobin reducing activity' (MRA) (Bekhit and Faustman 2005). The rate of metmyoglobin reduction is considered by many researchers to be a key determinant of meat colour stability (Faustman and Cassens 1990, Mancini and Hunt 2005), however its importance remains controversial (Ledward 1985, Echevarne, Renerre et al. 1990, Madhavi and Carpenter 1993, Bekhit, Geesink et al. 2001). Only a few studies have correlated MRA with meat colour stability (Hutchins, Liu et al. 1967, Ledward 1972, Sammel, Hunt et al. 2002, Bekhit, Geesink et al. 2003) while a study in lamb loin found no correlation between MRA and meat colour (Bekhit, Geesink et al. 2001).

2.4.3 Achromatic factors

Attributes that influence meat colour other than pigment are known as achromatic factors and these are mainly physical in nature. Light entering a meat surface can be either transmitted, reflected, refracted, diffracted or absorbed (Hughes, Oiseth et al. 2014). Structures with different refractive indices are present within meat, such as proteins, cell membranes and fat particles, that can be in gaseous, liquid or solid phases. The refractive index is the ratio of the velocity of light passing through a medium compared to a vacuum and this depends on the wavelength of light.

Factors in meat such as pH, intramuscular fat or marbling, collagen content, sarcomere length, protein denaturation or mitochondrial density may therefore influence light

reflection from a meat surface (Swatland 2012). Ultimate pH is the most well recognised achromatic factor influencing meat colour (Swatland 2004). The direction that meat is cut also influences achromatic light absorption, with transversely cut muscle fibres reflecting less light and thus appearing darker than fibres cut longitudinally (Swatland 2012, Hughes, Oiseth et al. 2014). The overall extent that achromatic absorption of light influences the bloomed colour of lamb meat and its stability is difficult to ascertain, as meat colour research has been predominantly focused on myoglobin biochemistry.

2.5 Factors influencing meat colour

2.5.1 Muscle factors

Muscle traits can influence myoglobin redox dynamics and therefore meat colour. Factors such as muscle fibre type and myoglobin concentration are relatively fixed in an animal, though can be influenced by genetotypic factors, while other factors such as MRA or meat pH may be readily influenced by external factors.

2.5.1.1 Muscle fibre type

Muscle fibres may be classified based on several physical and metabolic properties, including differences in morphological traits, energy metabolism, contractile properties, metabolic properties and colour (Picard, Lefaucheur et al. 2002, Lee, Joo et al. 2010, Lefaucheur 2010). Muscle fibres are commonly classified based on the relative importance of oxidative and glycolytic metabolism into oxidative muscle fibres (type I), oxido-glycolytic fibres (type IIA) and glycolytic fibres (type IIX and IIB) (Ashmore and Doerr 1971, Peter, Barnard et al. 1972, Schiaffino and Reggiani 1996). Oxidative metabolism utilises glycogen, glucose, lipids, ketone bodies and amino acids in the mitochondria and has a high oxygen requirement (Lefaucheur 2010), while glycolytic metabolism rapidly converts glycogen into lactate with no oxygen requirement (Lefaucheur 2010). The speed of muscle fibre contraction increases from slow twitch oxidative type I, to type IIA, type IIX, through to the fast twitch glycolytic type IIB fibres (Schiaffino and Reggiani 1996).

Oxidative muscle fibres are densely vascularised to supply oxygen for oxidative phosphorylation, have high concentrations of mitochondria (Hoppeler 1985, Klont 1998), high lipid and myoglobin content (Essén-Gustavsson, Karlsson et al. 1994) and

are red in colour. Glycolytic fibres have fewer mitochondria, less blood vessels and are whiter in colour (Nelson and Cox 2005).

2.5.1.2 Muscle oxidative capacity

Muscle oxidative capacity is an important determinant of bloomed meat colour and its stability on retail display. Muscle oxidative capacity is determined by the relative proportions of oxidative and glycolytic fibres (Staron and Johnson 1993, Brandstetter, Picard et al. 1998), which is regulated by genetic, physiological and environmental factors (Nelson and Cox 2005). Muscles with high proportions of oxidative muscle fibres such as the *m. psoas major* and *m. gluteus medius* have high oxidative capacity, while muscles such as the *m. semimembranosus* have intermediate oxidative capacity, and muscles with high proportions of glycolytic muscle fibres such as the *m. longissimus dorsi* and *m. semitendinosus* have low oxidative capacity (Hunt and Hedrick 1977).

The relative proportions of muscle fibres will also determine the relative expression of oxidative and glycolytic enzymes in a muscle. Muscles with high oxidative capacity will express higher levels of oxidative enzymes such as isocitrate dehydrogenase (ICDH, EC 1.1.1.41) (Hocquette, Ortigues-Marty et al. 1998). Muscles with a low oxidative capacity will express higher levels of glycolytic enzymes (Peter, Barnard et al. 1972). The relative expression of oxidative and glycolytic enzymes can therefore be used as an indicator of oxidative capacity in lamb muscle (Gardner, Pethick et al. 2006, Gardner, Hopkins et al. 2007, Lefaucheur 2010).

Muscle oxidative capacity impacts lamb meat colour by influencing the bloom depth and metabolic factors including ROS production and meat pH. High muscle oxidative capacity reduces bloom depth (Monin and Ouali 1992) due to increased myoglobin concentration physically obstructing oxygen diffusion, increased availability for oxygen

binding and due to increased oxygen utilisation with mitochondrial metabolism (King, Shackelford et al. 2010). The reduced redness and darkness caused by the reduced bloom depth in meat with high oxidative capacity may be offset to a certain extent by a concomitant increase in oxymyoglobin concentration and potentially increased redness associated with increased myoglobin availability to bind oxygen.

Muscles with high oxidative capacity are prone to more rapid meat browning on display than more glycolytic muscles (O'Keeffe and Hood 1982, Renerre and Labas 1987). Different muscles from the same carcass possess a wide range of colour stabilities (Hunt 1980) due largely to differences in the oxidative capacity of different muscles. Muscle type is the major factor controlling the rate of discolouration of beef muscle, accounting for almost half of the variance in colour stability (Hood 1980). The increased rate of browning in more oxidative muscles relates to the reduced bloom depth and to increased production of ROS that trigger myoglobin oxidation. These factors outweigh any associated increase in MRA and thus metmyoglobin reduction in more oxidative muscles (Echevarne, Renerre et al. 1990).

2.5.1.3 Mitochondrial activity and oxygen consumption rate

Muscle oxidative capacity is underpinned by mitochondrial concentrations within muscle fibres (Klont, Brocks et al. 1998). Muscle oxidative capacity is thus closely associated with mitochondrial activity and oxygen consumption in meat, which have similar influences on meat colour. Mitochondrial activity out-competes myoglobin for oxygen, reducing the bloom depth (Monin and Ouali 1992) and reducing meat lightness and redness (Ramanathan, Konda et al. 2009). Increased mitochondrial activity also increases production of ROS that trigger myoglobin oxidation (Sammel, Hunt et al. 2002, AMSA 2012), reducing meat colour stability. Atkinson and Folett (1973) found that oxygen consumption was positively related to the rate of browning in lamb, beef

and pork. Lamb meat had the highest oxygen consumption rate and thus the worst colour stability of these species. Differences in colour stability between species has been related to different rates of muscle oxygen consumption (Klont 1998).

Many studies have focused on the role of mitochondria and oxygen consumption in muscle *post-mortem* (Bendall and Taylor 1972) and have identified a number of factors influencing oxygen consumption rate including species, breed, muscle, pH, temperature, rigor temperature, electrical stimulation and meat age. High meat temperature or pH increase mitochondrial activity and thereby the rate of oxygen consumption, while increased meat ageing, rigor temperature and electrical stimulation reduce the oxygen consumption rate of meat. How long mitochondria continue to metabolise oxygen in muscle *post-mortem* has not been clearly defined, though intact mitochondria having been isolated from meat up to 60 days *post-mortem* (Tang, Faustman et al. 2005).

2.5.1.4 Isocitrate dehydrogenase (ICDH) activity

ICDH is a crucial enzyme in the oxygen-dependant citric acid cycle of mitochondria. Oxidative type I muscle fibres therefore have a greater expression ICDH (Peter, Barnard et al. 1972, Brandstetter, Picard et al. 1998) than more glycolytic fibre types. Given its high correlation with oxidative metabolism, ICDH activity has been used as a marker of oxidative capacity in lamb muscle (Gardner, Pethick et al. 2006).

ICDH activity in meat varies with production factors such as animal age, sex and genotype. ICDH activity increases with animal age (Brandstetter, Picard et al. 1998, Jurie, Martin et al. 2005, Gardner, Hopkins et al. 2007) and maturity (Schreurs, Garcia et al. 2008), in line with the increasing proportion of oxidative muscle fibres in older, more mature animals (Brandstetter, Picard et al. 1998). ICDH activity varies slightly between lamb sexes and more substantially between lamb breeds (Gardner, Hopkins et al. 2007). Genotypes expressing higher levels of IMF have been associated with higher

ICDH activity (Pethick, Hopkins et al. 2005), likely due to the positive association between muscle oxidative capacity and lipid content in muscle fibres (Picard, Lefaucheur et al. 2002, Hocquette, Gondret et al. 2010).

The effect of ICDH activity on lamb meat colour has not been demonstrated, however given its association with muscle oxidative capacity, ICDH activity is likely to influence the bloomed colour of lamb meat and its stability on display.

2.5.1.5 Myoglobin concentration

Myoglobin concentration directly influences bloomed colour and potentially influences colour stability through its association with muscle oxidative capacity. Though considered an endogenous and relatively fixed muscle trait, myoglobin concentration changes with animal age/maturity and is very heritable, with 85% of the variation in beef myoglobin content explained by genetic factors in beef (King, Shackelford et al. 2010, King, Shackelford et al. 2012).

Myoglobin concentration is an important determinant of bloomed meat lightness and redness, which are important to consumer acceptability of fresh meat (Khliji, Van de Ven et al. 2010). Myoglobin concentration is negatively correlated with L^* in beef (McKenna, Mies et al. 2005) as myoglobin pigments absorb visible wavelengths of light, reducing the amount of reflected light and the perceived lightness of the meat. Myoglobin concentration is positively associated with meat redness, likely due to increased myoglobin availability to bind oxygen and form oxymyoglobin and potentially due to the association between myoglobin and red oxidative muscle fibres. MacDougall (1982) found that the oxymyoglobin concentration of bovine muscle was negatively associated with meat L^* and hue, and positively associated with chroma. MacDougall et al. (1973) found that small increases in myoglobin in veal meat could produce changes in colour that have considerable consequences commercially, whereas

similar or greater increases in myoglobin concentration in mature beef may not be noticed.

The influence of myoglobin concentration on meat colour stability is less well established and is difficult to clearly separate from an associated influence of muscle oxidative capacity. McKenna (2005) found no relationship between myoglobin content and meat browning in 19 different beef muscles, while King et al. (2010) found that breeds with high colour stability generally had lower myoglobin concentrations compared to other breeds. This finding likely reflects as associated reduction in oxidative capacity associated with lower myoglobin concentrations. O'Keefe and Hood (1982) speculated that muscles containing lower myoglobin content were less stable in colour because their myoglobin was oxidised at a greater frequency to maintain normal cellular respiration. Garry and Mammen (2007) noted that one of myoglobin's functions *in vivo* is to serve as a scavenger of ROS, which may counteract the negative association between myoglobin concentration and colour stability anticipated due to increased muscle oxidative capacity.

Production factors such as age, sex and genotype that influence myoglobin concentration will also impact meat colour. Myoglobin increases with animal age and weight (Jacob, D'Antuono et al. 2007), causing older animals to produce darker meat (Sookhareea, Taylor et al. 1995). Increased in myoglobin concentration in older animals (Pethick, Hopkins et al. 2005, Gardner, Hopkins et al. 2007) is linked to an increasing proportion of type I muscle fibres and a more oxidative muscle metabolism in older animals (White, McGavin et al. 1978, Suzuki and Cassens 1983, Brandstetter, Picard et al. 1998, Greenwood, Harden et al. 2007). In sheep, Gardner et al. (2007) demonstrated that myoglobin concentration doubled in all muscles from 4 to 22 months of age. The largest increase in myoglobin occurred in these lambs from 4 to 8 months of age.

Numerous other studies have demonstrated the increase in myoglobin concentration with age and maturity in sheep (Ledward and Shorthose 1971, Pethick, Hopkins et al. 2005, Gardner, Hopkins et al. 2007, Jacob, D'Antuono et al. 2007). Older and more mature lambs produce redder meat due to increased myoglobin content (Hopkins, Stanley et al. 2007). The influence of animal sex on myoglobin concentration is less clear. Gardner et al. (2007) found no association ($P > 0.05$) between lamb sex and myoglobin concentration. However, earlier work found ewe lambs to have 10% more myoglobin than wether lambs (Ledward and Shorthose 1971), which have been shown to have a reduced proportion of glycolytic muscle fibres (Greenwood, Harden et al. 2007). The impact of genotype on myoglobin concentration is also unclear, some studies showing lower myoglobin concentrations in Merino lambs compared to faster growing meat breeds (Gardner, Kennedy et al. 1999, Gardner, Hopkins et al. 2007) while other studies showed no difference in myoglobin concentrations between breeds (Hopkins, Hegarty et al. 2005). Better understanding influences on muscle myoglobin concentration has the potential to improve the bloomed colour of lamb meat on display.

2.5.1.6 Iron and zinc concentration

Lamb meat contains high levels of certain nutrients shown to be important for human health, including iron and zinc (Pannier, Ponnampalam et al. 2010). Red meat is recognised as one of the best sources of iron and zinc because of their high bioavailability in meat compared to plant sources (Linder 1991). Australian lamb is currently marketed as a ‘good’ source of iron and zinc (Pannier, Ponnampalam et al. 2010, Pannier, Pethick et al. 2014).

The influence of muscle iron and zinc concentration on lamb meat colour is unclear. Iron is likely to impact meat colour due to its association with myoglobin concentration and muscle oxidative capacity. Iron is central to the heme group that is found in

myoglobin as well as haemoglobin (Nelson and Cox 2005). Myoglobin and haemoglobin are crucial to oxygen delivery for muscle metabolism, linking muscle iron concentration to muscle oxidative capacity. Warner, Kearney, Hopkins and Jacob (2017) reported that increasing iron concentration reduced the retail shelf-life of over-wrapped lamb meat by ~0.75 days.

Muscle zinc concentration has also been linked to oxidative capacity. High iron and zinc concentrations are present in muscles with high oxidative capacity (Cassens, Hoekstra et al. 1967, Kondo, Kimura et al. 1991, Pearce, Pannier et al. 2009), and are associated with increased myoglobin and ICDH activity (Pannier, Pethick et al. 2014), iron more closely than zinc. Iron and zinc concentrations have also been positively associated with IMF content and to be influenced by production factors such as lamb age, sex and genotype (Ono, Berry et al. 1984, Lin, Cross et al. 1988, Pannier, Ponnampalam et al. 2010). Iron and zinc concentrations increase with lamb age (Ono, Berry et al. 1984, Lin, Cross et al. 1988, Pannier, Ponnampalam et al. 2010), have been reported to be higher in male lambs in some studies (Bellof, Most et al. 2007) and female lambs in others (Pannier, Pethick et al. 2014), and are impacted upon by animal breed (Underwood 2012). Given their association with myoglobin and muscle oxidative capacity, it is likely that zinc and particularly iron will influence the display colour of lamb meat.

2.5.1.7 Metmyoglobin Reducing Activity

Meat contains limited amounts of natural enzymes collectively referred to as metmyoglobin reducing activity (MRA) that can reduce oxidised myoglobin (metmyoglobin, Fe^{3+}) to deoxymyoglobin (Fe^{2+}). However, there have been inconsistent reports and diverging conclusions regarding the importance of MRA to meat colour stability (Bekhit and Faustman 2005). Some researchers have reported no relationship between MRA and meat colour stability (Renerre and Labas 1987, Echevarne, Renerre

et al. 1990, Lanari and Cassens 1991, Reddy and Carpenter 1991) while others researchers assert that MRA is the primary factor governing metmyoglobin accumulation and thus regulating meat colour stability (Ledward and Shorthose 1971, Ledward 1985, AMSA 2012). While a number of factors such as temperature and pH have been identified that affect MRA and that may in turn influence lamb meat colour on display (Bekhit and Faustman 2005), (Reddy and Carpenter 1991), Bekhit et al. (2001) found no association between MRA and colour stability in lamb loin muscle. Therefore while acknowledging its potential importance to red meat colour stability; MRA is not a focus of this study.

2.5.1.8 Meat pH

pH is a measure of the hydrogen ion concentration and thus the acidity or alkalinity of a solution. Meat pH is possibly the single most important factor affecting fresh meat colour development and its stability over retail display (AMSA 2012). The pH of muscle in a live animal is almost neutral at 7.1. *Post-mortem* glycolysis produces lactic acid which causes muscle pH to decline. The rate and extent of *post-mortem* glycolysis will determine the cessation of pH decline and the ultimate pH reached in meat. Factors including nutrition, pre-slaughter stress, muscle fibre type and chilling temperatures influence the extent of *post-mortem* glycolysis and therefore the ultimate pH of meat. pH decline has largely ceased within 24 hours in rapidly chilled lamb carcasses, therefore pH measured at 24 hours *post-mortem* may be used to approximate ultimate pH in lamb meat.

Meat pH influences key processes influencing meat colour - *post-mortem* mitochondrial activity and oxygen consumption, the depth of oxygen penetration, MRA and the auto-oxidation of myoglobin (Faustman and Cassens 1990). However, there are substantial inconsistencies in the reported impact of pH on meat colour, particularly colour

stability. The negative effects of high pH on the bloomed colour of beef has been well established due to the commonly encountered problem of 'dark cutting' beef. High pH favours increased metabolic enzyme activity, increasing oxygen consumption, outcompeting myoglobin for oxygen and reducing the bloom depth of meat (Kropf 1993). By reducing oxygen penetration and availability to bind with muscle myoglobin, the proportion of oxymyoglobin: deoxymyoglobin in a meat surface is reduced, causing high pH meat to appear darker and less red (Hopkins, Lamb et al. 2013).

Muscle pH influences the water binding nature of proteins and thus directly affects the physical structure of meat and its light reflecting properties (Andrés-Bello, Barreto-Palacios et al. 2013). A high pH causes little to no shrinkage of the myofilament lattice, reducing the differences in the refractive index of the myofibrils and sarcoplasm, increasing light absorption and reducing the perceived lightness of meat (Adzitey and Nurul 2011). The more closed muscle structure in high pH meat also reduces the depth of oxygen penetration and thus the bloom depth of meat.

The impact of meat pH on retail colour stability remains unclear. Several studies have shown that pH over a limited range (5.6-5.8) is not a major determinant of meat colour stability (Ledward 1970, Hood 1980). McKenna (2005) also found a low correlation between pH and meat discolouration. Other studies such as Tang et al. (2005) reported that low pH improves meat colour stability. Tang et al. (2005) found that mitochondrial oxygen consumption was reduced in vitro at pH 5.6 compared to 7.2, reducing the conversion of oxymyoglobin and deoxymyoglobin into metmyoglobin and reducing browning. A low meat pH may also improve colour stability by reducing oxygen consumption, thereby increasing the bloom depth and increasing the time taken for metmyoglobin accumulation to cause visible meat browning (Lawrie 1958).

Other research suggests that increasing pH improves colour stability, as high pH protects myoglobin from oxidation and low pH favours metmyoglobin formation (Brown and Mebine 1969, Hunt, Sørheim et al. 1999, O'Grady, Monahan et al. 2001, Gutzke and Trout 2002, AMSA 2012). Several researchers report that myoglobin oxidation and browning was inhibited as pH increased from 5.6 to 8.5 (Shikama and Sugawara 1978, Yin, Faustman et al. 1993). Ledward (1985) concluded that as oxidation reduces and reducing activity is believed to increase with increasing pH, that higher pH muscles would have improved colour stability. Therefore, while pH is widely acknowledged to be an important influence of red meat colour stability, its impact remains disputed. The majority of research on meat pH and colour has been conducted in beef, thus the optimal meat pH to improve the colour stability of lamb meat remains unclear.

2.5.1.9 Lean meat yield and muscling

Lean meat yield is the proportion of lean muscle or meat derived from a carcass relative to fat and bone, and is a key productivity driver in the lamb meat supply chain. Lean meat yield has rapidly increased over the last 15 years in Australia via genetic selection of lambs for rapid muscle growth (Gardner, Williams et al. 2010). Selection for muscling has been shown to promote increased expression of more glycolytic type IIB muscle fibres in a number of domestic species including lambs (Wegner 2000, Greenwood, Gardner et al. 2006), resulting in less oxidative muscle tissue with a paler (whiter) appearance (Gardner, Pethick et al. 2006). Selection for lean meat yield using Australian Sheep Breeding Values (ASBVs) for Post-weaning Eye Muscle Depth (PEMD) and Post-weaning Fat Depth (PFAT) has reduced myoglobin and ICDH activity in the loin muscle of lambs (Kelman, Pannier et al. 2014), supporting a shift to more glycolytic muscle fibres. Due to these associations with muscle oxidative capacity

and myoglobin concentration, selection for lean meat yield or muscling is likely to impact the colour of lamb meat of retail display.

2.5.1.10 Intramuscular fat (IMF)

IMF is the adipose tissue contained within muscles. Triacylglycerol is the major lipid component of IMF, that is comprised of adipocytes embedded in a connective tissue matrix (Murray, Granner et al. 2004). IMF concentration is positively associated with flavour, juiciness, tenderness and overall liking in lamb meat (Hopkins, Hegarty et al. 2006, Pannier, Pethick et al. 2014). Meat with low IMF is perceived by consumers to be dry and less tasty (Channon, Reynolds et al. 2001, McPhee, Hopkins et al. 2008). Acceptable levels of IMF in lamb are cited as 3-4 % by Savell and Cross (1988) though Hopkins, Hegarty et al. (2006) demonstrated that a minimum of 5% IMF is required to achieve an average sensory score for overall liking of sheep meat.

The portion of IMF visible at a meat surface is known as marbling. Marbling is used by consumers as an indicator of eating quality, though high levels of IMF can deter consumers due to health concerns about the level of saturated fat intake (Bünger, Navajas et al. 2009). Visual assessment of marbling is used to grade beef carcasses under the MSA system (Polkinghorne, Watson et al. 1999), however marbling is less visible in lamb meat.

IMF concentration is associated with muscle oxidative capacity and thereby may influence meat colour. Oxidative muscle fibres are associated with higher levels of IMF (Picard, Lefaucheur et al. 2002, Hocquette, Gondret et al. 2010), though the association between IMF and muscle fibre type composition is disputed (Lefaucheur 2010). Fiems, Campeneere et al. (2000) reported a negative correlation between IMF and meat *L**, suggesting that a reduction in IMF may lighten meat colour via an associated increase in glycolytic muscle fibres. Fiems et al. (2000) reported no genetic correlations between

IMF, *L**, *a** and *b**, though reported phenotypic correlations between IMF and *a** of 0.25, and between IMF and *L** of -0.06. The direction of these phenotypic relationships support an association between IMF and myoglobin concentration. IMF may also influence meat colour via its association with the triacylglycerol content (Hocquette, Jurie et al. 2003) and lipid oxidation (Giddings and Hultin 1974) in meat.

2.5.1.11 Lipid oxidation

Lipid oxidation is linked to colour stability in red meats. Lipid oxidation generates reactive secondary products that are responsible for off-odours and off-flavours in meat (Pearson, Love et al. 1977) and that also accelerate myoglobin oxidation and browning in meat (Faustman, Liebler et al. 1999). Lipid oxidation and myoglobin oxidation appear to be linked processes, with oxidation of one leading to the formation of chemical species that can exacerbate the oxidation of the other (Faustman, Sun et al. 2010). Significant support of this link has been provided by the antioxidant mediation of both processes (Faustman, Sun et al. 2010). For example it is well established that the lipid-soluble antioxidant vitamin E inhibits lipid oxidation in meat from different livestock species (Faustman 2004). The observation that vitamin E also delayed beef discolouration, a process based on oxidation of a water-soluble protein, therefore provides evidence of a strong link between these processes (Faustman, Sun et al. 2010).

A variety of intrinsic properties and processing factors can predispose meat to lipid oxidation and thereby browning. Strategies to inhibit lipid oxidation will minimise rancidity and improve meat colour stability (Faustman, Sun et al. 2010). Lipid oxidation increases dramatically when the number of unsaturated (double) bonds increases, meaning that poly-unsaturated fatty acids (PUFAs) are oxidised at a much faster rate than mono-unsaturated fatty acids (MUFAs) (Li and Liu 2012). Monogastric muscles contains relatively more unsaturated fatty acids within triacylglyerols and are thereby

more prone to lipid oxidation than ruminants. In beef and lamb production, increasing PUFA concentration in meat by feeding PUFA-enriched feeds is less effective than in monogastrics as unsaturated fatty acids can be hydrogenated in the rumen (Li and Liu 2012).

2.5.1.12 Antioxidants

Antioxidants are cellular components that have the ability to neutralize the harmful effects of free radicals or free radical generating species. In a physiological cell, oxidation is maintained in a sort of equilibrium due to antioxidant activity. In times of oxidative stress, antioxidant systems become saturated and cellular damage results. *Post-mortem* muscle is undergoing *post-mortem* stress, thus oxidative load is increased and the activity of antioxidants is altered to a degree that is not completely understood.

There are different types of antioxidants that vary in their degree of antioxidant potential. Antioxidants may be proteins, enzymes, metabolites and vitamins, and are usually essential requirements in an animal's diet. In meat production, antioxidants can be supplemented to animals or applied to meat *post-mortem* to minimise lipid and myoglobin oxidation (Faustman, Sun et al. 2010). Feeding animals with plant extracts rich in antioxidant compounds can also improve meat colour stability (Suman, Hunt et al. 2014).

2.5.1.13 Vitamin E

Vitamin E is the most commonly used antioxidant in meat quality research (Jose 2011). Vitamin E is lipid soluble and thus membrane-bound antioxidant that functions to protect lipids such as PUFAs and myoglobin from oxidation (Faustman, Chan et al. 1998).Vitamin E has a variety of naturally occurring forms, alpha-tocopherol being the most biologically active form and comprising about 90% of all tocopherols in animal

tissue (Wolf, Wolf et al. 1998). Supplementing animal diets with Vitamin E increases α-tocopherol deposition in adipose tissue, live and muscle (Machlin 1984), (Jensen, Lindholm et al. 1989). Muscle deposition of α–tocopherol prevents lipid and pigment oxidation by acting directly on cell membranes (Higgins, Kerry et al. 1998). Lanari et al. (1994) showed that vitamin E stabilises the oxymyoglobin complex by enhancing myoglobin oxygenation and decreasing oxidation, while other researchers suggest that vitamin E reduces myoglobin oxidation indirectly through its inhibition of lipid oxidation (Faustman, Chan et al. 1998).

Dietary vitamin E supplementation improves the colour stability of beef (Faustman, Cassens et al. 1989, Liu, Lanari et al. 1995, Smith, Morgan et al. 1996, Faustman, Chan et al. 1998, Lynch, Kerry et al. 1999, Stubbs, Morgan et al. 2002, Robbins, Jensen et al. 2003) and lamb meat (Wulf, Morgan et al. 1995, Guidera, Kerry et al. 1997, Strohecker, Faustman et al. 1997, Lauzurica, de la Fuente et al. 2005). Vitamin E accumulation in muscle tissue is muscle-dependent (Arnold, Arp et al. 1993a), for example vitamin E concentrations having been found to be consistently higher in the *m. gluteus medius* than the *m. longissmus lumborum*. This may relate to muscle oxidative capacity, Salviati et al. (1980) reporting that type I muscle fibres contain higher vitamin E concentrations than type II muscle fibres. Type I muscle fibres have greater capillary distribution and contain more numerous small mitochondria between myofibrils than type II fibres (Porter and Palade 1957), potentially meaning an increased supply and capacity to store vitamin E in muscle (Liu, Lanari et al. 1995). However, studies have concluded that inherent differences in colour stability among muscles cannot be attributed to differences in vitamin E concentration nor changed with vitamin E supplementation (Faustman, Chan et al. 1998, Jacob, D'Antuono et al. 2007).

The muscle, rate and duration of vitamin E supplementation influence muscle vitamin E concentration (Arnold, Arp et al. 1993a). Vitamin E concentrations of 3-4 mg/kg muscle are needed to positively influence meat colour stability (Faustman, Cassens et al. 1989). The rate of supplementation required to achieve this concentration depends on the duration of supplementation and muscle (Figure 2-8). Vitamin E can be supplemented at 400IU/kg ration for 1-2 weeks or at 150 IU/kg ration for 2.5 - 4 weeks to achieve a vitamin E concentration of 3.5mg/kg in the *m. semimembranosus* and *m. semitendinosus* (Figure 2-8) (Jose 2011). Only 15 - 40 IU of vitamin E is required to maintain the health of sheep (McDowell 1989).

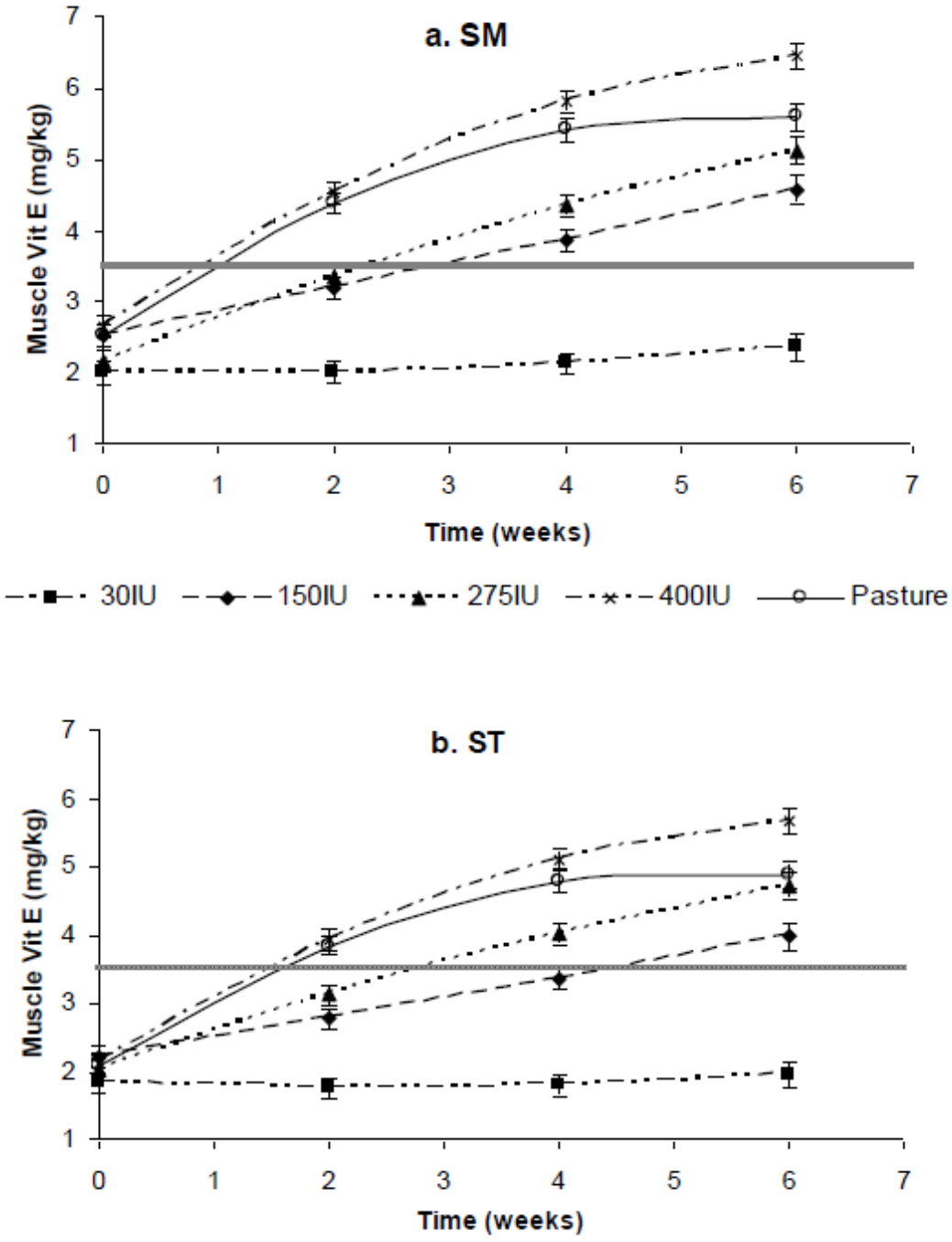

Figure 2-8 Muscle vitamin E (Vit E) concentration with dietary vitamin E supplementation.
Muscle vitamin E was measured in the a) Semimembranosus and b) Semitendinosus fortnightly by biopsy as lambs were supplemented with 30, 150, 275 and 400 IU of dietary vitamin E daily (Jose 2011).
Grey bolded lines represent the muscle vitamin E threshold of 3.5mg/kg required to positively influence meat colour stability (Faustman, Cassens et al. 1989).

The positive effect of increasing muscle vitamin E on meat colour stability has a threshold. Arnold, Scheller, Arp, Williams and Schaefer (1993b) found no additional

benefit when vitamin E was supplemented at 1290 IU/day compared to 360 IU/day in 7 day aged beef. The higher dose did however improve retail colour stability after 21 days ageing (Arnold, Scheller et al. 1993b). Faustman et al. (1999) reported that vitamin E concentrations in beef reduced with meat storage, suggesting muscle vitamin E concentration or antioxidant capacity decreases with meat ageing.

Unlike meat colour stability, the influence of vitamin E on the bloomed colour of lamb meat is unclear. Dietary vitamin E intake has been linked to improved bloomed meat colour, with Lanari, Cassens, Schaefer and Scheller (1994) reporting that vitamin E supplementation enhanced myoglobin oxygenation, increasing the % of oxymyoglobin after blooming. This may underpin research reporting that vitamin E supplementation increases lamb meat lightness, redness (Arnold, Scheller et al. 1992, Pearce, Masters et al. 2005), and is preferred by consumers throughout retail display (Wulf, Morgan et al. 1995).

2.5.2 Processing and retail factors

Different strategies are used across the red meat supply chain that may influence meat display colour. Strategies include carcase electrical stimulation, meat ageing, packaging and display conditions at retail.

2.5.2.1 Chilling rate and pH decline

The rate of carcass chilling and pH decline has been reported to influence meat colour (Kim, Luc et al. 2013). Modern carcass chilling practises have focused on speeding up the chilling process using refrigerated air to reduce microbial growth and evaporative weight loss (Adzitey and Nurul 2011). Faster chilling rates produce a rapid drop in the carcass temperature and thereby reduce the rate of pH decline. The effect and importance of carcass chilling and rate of pH decline on meat colour remains unclear

however. Kim et al. (2013) reported that slow chilled aged lamb loin browned more rapidly on retail display, while other studies have linked faster chilling rates with increased meat browning on display (Ledward 1985, Kim and Hunt 2011, Rosenvold and Wiklund 2011) due to myoglobin denaturation. Warriss (2000)reported that rapid chilling improved fresh meat colour, while Hopkins et al. (2013) reported that rapid chilling reduced lamb loin *a** and R630/R580 on display. Any improvement in bloomed meat colour with slower chilling and faster rates of pH decline is limited by low pH and high temperature conditions affecting mitochondrial integrity and functionality (England, Scheffler et al. 2013), damaging proteins and negatively influencing meat colour. Associated colour changes (typically increased *L** and reduced chroma) are due to combined effects on oxygen penetration depth and the scattering of light from denatured sarcoplasmic proteins (Swatland 1995).

2.5.2.2 Electrical stimulation

Carcasses are commonly electrically stimulated in abattoirs to ensure that carcasses meet the target rate of pH decline (pH/carcass temperature window) that produces optimal meat tenderness (George, Bendall et al. 1980). Electrical stimulation has been linked to increased lightness and brightness of bloomed meat (Savell, Smith et al. 1978, Sleper, Hunt et al. 1983, Unruh, Kastner et al. 1986, Eikelenboom, Hoving-Bolink et al. 2000). The improvement in bloomed colour following electrical stimulation (Moore and Young 1991) may relate to loosening of the muscle lattice structure, causing more light scattering and a lighter perceived meat colour (Unruh, Kastner et al. 1986).

Electrical stimulation has also been linked to meat colour stability. Ledward (1985) reported that stimulated muscle was more prone to metmyoglobin formation and meat browning, potentially due to a decrease in metmyoglobin reducing capacity (Sleper, Hunt et al. 1983). However Ledward (1985) also reported that electrical stimulation will

have minimal effect on their colour stability in muscles that are inherently colour stable and able to be chilled rapidly due to their exterior position on the carcass, such as the *m. latissimus dorsi.* Other studies have reported improved meat colour stability following electrical stimulation. Moore and Young (1991) and Lindhal, Karlsson, Lunstrom and Andersen (2006) reported an increased colour shelf life in electrically stimulated lamb and pig meat, while other studies showed an improvement in colour only over the first days of display (Sleper, Hunt et al. 1983, Ledward 1985, Unruh, Kastner et al. 1986). Jose (2011) found that medium voltage electrical stimulation had a detrimental effect on the colour stability of lamb meat after 5 days of ageing but not after 10 days ageing. The different reported effects of electrical stimulation on colour stability may be due to different stimulation devices used. Generally, high voltage electrical stimulation systems have been reported to negatively impact meat colour stability (Moore and Young 1991), while medium and low voltage systems have been reported to have little effect on colour stability (Ledward, Dickinson et al. 1986, Toohey and Hopkins 2006, Jacob, D'Antuono et al. 2007).

2.5.2.3 Meat storage (ageing)

Post-mortem ageing or storage of meat is widely employed globally to improve tenderness and palatability (Suman, Hunt et al. 2014). Most lamb and beef is retailed in grocery stores following 5 - 21 days ageing in a vacuum bag (‘wet aged’). However vacuum packaged lamb meat may be stored at low temperatures (-1.5 to 0°C) for up to 12 weeks prior to retail display (MLA 2005). Exporters such as Australia and New Zealand commonly age lamb for 8 – 9 weeks at -1.5°C for shipping to distant markets (Kim, Stuart et al. 2012). Meat tenderness and flavour are the two major attributes improved with ageing, however ageing conditions also influence cellular mechanisms such as mitochondrial activity (Ledward 1985, Tang, Faustman et al. 2005) and thereby

the bloomed colour of red meat and its stability over retail display (Suman, Hunt et al. 2014).

Mitochondrial activity reduces with meat ageing (Tang, Faustman et al. 2005), therefore oxygen consumption rates also reduce with meat ageing due to declining mitochondrial respiratory activity of enzymes (Bendall & Taylor, 1972; Ledward, 1985; O'Keefe & Hood, 1982). Meat vacuum-aged for several weeks blooms more rapidly and intensely than fresh meat due to this loss of activity of the oxygen-consuming enzymes (Ledward 1992). Reduced oxygen consumption increases the availability of oxygen to bind with myoglobin in aged meat, thereby allowing greater blooming (Kim, Frandsen et al. 2011). Structural changes in muscle proteins during ageing influence meat lightness, several studies reporting increased meat lightness with ageing (MacDougall 1982, Boakye and Mittal 1996, Gašperlin, Žlender et al. 2001). Meat redness and chroma increase with meat ageing due to increased myoglobin oxygenation and bloom depth (Bendall and Taylor 1972, Atkinson and Follett 1973, Ledward 1992, Mancini and Ramanathan 2014). However, the effect of ageing on bloomed meat colour appear to vary depending on the duration of meat ageing. Lee et al. (2008b) found that ageing beef m. *gluteus medius* steaks for up to 35 days did not influence meat lightness, while 7-21 days ageing optimised meat redness. Additionally, Hopkins et al. (2013) reported that short-ageing lamb for 1 or 5 days had no effect on the display colour (R630/R580 or *a**) of lamb loin.

The improved bloomed colour with meat ageing is offset by reduced colour stability on retail display. Suman et al. (2014) demonstrated in beef *m. longissimus* that initial red colour intensity increased with ageing up to 45 days, though colour stability decreased. Studies in lamb reported that vacuum-ageing increased meat redness but reduced colour stability over a three day display (Ponnampalam, Trout et al. 2001, Ponnampalam,

Butler et al. 2013). The influence of ageing on meat colour stability also depends on the duartion of storage. It is well established that very fresh meat (up to 3 days post-slaughter) will brown more rapidly than meat aged for several days (AMSA 2012). However researchers have shown that prolonged ageing reduces colour stability in beef and lamb meat (Moore and Young 1991, Wulf, Morgan et al. 1995, Eikelenboom, Hoving-Bolink et al. 2000). Ledward et al. (1986) found that beef on display browned more rapidly following 33 days ageing than 5 days ageing, while King et al. (2012) found that increasing wet-ageing of beef longissimus steaks from 14 to 35 days also reduced subsequent colour stability on retail display. Moore and Gill (1987) reported that vacuum ageing reduced colour stability of lamb meat during display, the magnitude of this effect depending on storage time and temperature.

The reason that aged meat has reduced colour stability on retail display is unclear. Mitochondrial activity and oxygen consumption are reduced and thus the bloom depth is increased in aged meat, factors that are associated with improved colour stability. Increased lipid oxidation with meat ageing (Eikelenboom, Hoving-Bolink et al. 2000, Ismail, Lee et al. 2008), increased meat pH (Callejas-Cárdenas, Caro et al. 2014) and/or a reduction in MRA (Hood 1980) may account for the reduced colour stability of aged meat.

2.5.2.4 Meat packaging

The quality and shelf life of refrigerated meat are strongly influenced by the packaging and storage conditions on retail display. The short shelf life of packaged lamb meat is one of the key problems with its commercialisation (Jeyamkondan, Jayas et al. 2000). The two most important factors limiting the shelf life of fresh meat are colour and microbial growth, with lipid oxidation the third most important factor (Lauzurica, de la Fuente et al. 2005). Meat packaging systems thereby aim to improve colour stability,

reduce microbial growth and limit lipid oxidation in displayed meat. Under aerobic packaging conditions, lipid oxidation does not limit meat storage life because it occurs at a much slower rate than discolouration or microbial growth (Jakobsen and Bertelsen 2000).

Vacuum packaging of meat (particularly primal and sub-primal cuts) is used regularly in the industry to extend meat shelf life (Walsh and Kerry 2002). Under the anaerobic conditions created by vacuum packaging the growth of aerobic meat-spoiling bacteria is prevented, however the red colour of meat is also prevented due to a lack of oxygen (Jeyamkondan, Jayas et al. 2000). Vacuum packaging is commonly used to store or age meat for days or weeks before the meat is removed, cut for display and packaged under aerobic conditions (McMillin 2008). The simplest and traditionally the most common form of meat packaging for retail display is overwrapping- where meat is packaged on disposable trays and wrapped with oxygen-permeable cling film. Though a very economic means of packaging, overwrapped meat has a limited shelf life in terms of colour. Overwrapped lamb meat has a colour shelf life of only 2 to 3 days at retail, before meat browning deters consumer purchase (Khliji, Van de Ven et al. 2010).

The colour shelf life of meat can be considerably increased using modified atmosphere packaging (MAP) (McMillin 2008). MAP is the removal and/or replacement of the atmosphere surrounding meat before sealing in impermeable materials. MAP plays an important role in beef and lamb retailing, improving meat colour stability and presenting the meat in an attractive manner to consumers. High-oxygen MAP maintains the bright red colour of fresh lamb and beef (Penney and Bell 1993), lamb meat retaining its premium bloomed colour for over 8 days under high oxygen MAP (Channon, Baud et al. 2005). High-oxygen MAP minimises the low oxygen tension conditions that favour metmyoglobin formation. High-oxygen MAP prevents microbial

growth of anaerobic pathogens (Ogrydziak and Brown 1982) while inclusion of moderate carbon dioxide levels (10 - 20%) inhibits the growth of aerobic bacteria (Ray 1996). Higher proportions of carbon dioxide (30%) will reduce meat colour stability (Silliker, Woodruff et al. 1977). The disadvantages of high-oxygen packaging include the expense of the packaging and an increase in lipid oxidation resulting in quality losses (Renerre and Labadie 1993).

2.5.2.5 Temperature

Temperature is the most important intrinsic factor influencing the storage life of fresh meat (Jeyamkondan, Jayas et al. 2000, AMSA 2012). Practices such as MAP or ageing are only effective when combined with low temperatures. Meat temperature is determined by the ambient air temperature and therefore temperature is also the most important extrinsic factor affecting meat discolouration that can be controlled by processing and storage conditions (Jeyamkondan, Jayas et al. 2000). Temperature influences the rate of oxygen diffusion (Rikert, Bressler et al. 1957), the activity of enzymes that utilise oxygen (Kropf 1993) and thus mitochondrial activity and oxygen consumption in meat. Temperature is therefore an important determinant of bloomed colour and particularly colour stability on retail display.

Lower storage temperatures increase the penetration of oxygen into meat due to reduced enzyme activity and mitochondrial oxygen consumption (Kropf 1993), making meat bloom more rapidly and extensively (AMSA 2012). Bloom depth is reduced in warmer meat as cellular enzyme systems out-compete myoglobin for oxygen. Low storage temperatures also discourage the disassociation of oxygen from oxymyoglobin (Rikert, Bressler et al. 1957), resulting in a greater proportion of oxymyoglobin: deoxymyoglobin and a brighter, lighter meat colour.

Colour stability is also maximised at low storage temperatures, as high temperatures accelerate the rate of myoglobin oxidation (Ledward 1985). The optimum temperature to maximise meat storage life is -1.5°C (Jeyamkondan, Jayas et al. 2000), oxymyoglobin pigments being most stable at -1 to 2°C (AMSA 2012). The oxidation rate of myoglobin increased 5 fold for every 10°C increase in temperature (Gutzke and Trout 2002). O'Keefe and Hood (1982) showed that beef stored at 10°C degrees is 2-5 x browner than meat stored at 0°C after 4 days of display. In the United States, the mean temperature of meat storage is 2°C, though the range is -2 to 6°C, meaning that on average, only 25% of potential storage life is actually achieved (Jeyamkondan, Jayas et al. 2000). The ambient temperatures produced in experimental simulated retail displays are likely to be lower and more consistent than those seen in retail display cabinets, which are often open to ambient conditions and undergo regular defrost cycles (Jacob, D'Antuono et al. 2014).

Freezing is an excellent method of preservation and offers several advantages including greater marketing flexibility, reduced equipment costs and improved sanitation (Jeyamkondan, Jayas et al. 2000). However frozen and thawed meat is generally considered to have inferior quality attributes (Kim, Luc et al. 2013), may appear less appealing (Moore and Young 1991) and have a reduced shelf life once thawed (Moore 1990). The palatability and storage life of frozen beef is limited primarily by lipid oxidation and surface discolouration (Chow, Ochiai et al. 1987, Lanari, Cassens et al. 1993). Reduced colour stability after freezing may relate to ice crystal formation and cell breakage causing increased interaction between the oxidative by-products of myoglobin and lipid oxidation (Chu, Huffman et al. 1987).

2.5.3 Production and genetic factors

A variety of production and genetic factors influence lamb meat colour. In general, these factors influence intrinsic muscle traits such as muscle fibre type, oxidative capacity or myoglobin concentration that in turn influence the display colour of lamb meat. Better understanding of the impact of these factors have on intrinsic muscle traits and thereby meat colour is needed to provide producers with strategies to reduce meat discolouration and associated economic losses.

2.5.3.1 Management practices

Management practices such as the intensity of production, frequency and nature of animal handling, feeding regimes and therapeutic procedures may influence lamb meat colour. These influences of management factors on meat colour remain unclear however, likely as the influences themselves are subtly and due to difficulty separating management practices from related influences on meat colour in research experiments. Pre-slaughter stress and feeding are important factors influencing meat pH. Stressed animals produce meat with a higher pH, darker colour and a dry appearance (Ashmore and Doerr 1971). Production factors such as the conditions prior to slaughter (transport, handling, mixing of animals) and the regularity of handling will influence animal stress, meat pH and retail colour. The plane of nutrition prior to slaughter will also influence factors such as vitamin E concentration and meat pH and thereby meat colour.

2.5.3.2 Nutrition

The influence of lamb nutrition on retail meat colour is not well understood. Finishing diets including milk, dry pasture, green pasture or grain/concentrate feeds have been shown to influence meat colour (Realini, Duckett et al. 2004, Pearce, Masters et al. 2005, Luciano, Monahan et al. 2009, Ramírez-Retamal and Morales 2014). Luciano et

al. (2009) reported that lambs fed a concentrate based diet produced meat with higher lipid and myoglobin oxidation than lambs fed fresh pasture. Gao et al. (2014) found a combination of pasture and feed-lot feeding reduced muscle oxygen consumption and improved the colour stability of lamb meat.

A myriad of different factors within a diet are likely to influence meat colour. Nutrition is an important determinant of meat pH, muscle antioxidant concentrations and fatty acid profiles, which all in turn influence retail meat colour. The antioxidant vitamin E is an important influence on meat colour and intramuscular vitamin E concentrations are determined by dietary intake. The influence of other antioxidants and nutritional factors on meat colour are less well established. Trace elements may influence the normal function of endogenous antioxidant systems (Petron, Raes et al. 2007) while minerals such as Cu, Mn, Zn, Se and Fe are important cofactors of antioxidant enzymes activities (Papas 1999) and may therefore influence meat colour.

Pro-oxidants in feed and their fatty acid profiles may influence meat colour (Leedle and Aust 1986, Agradi, Abrami et al. 1993). The proportions of PUFAs available in a feed influence the extent of lipid oxidation and thereby myoglobin oxidation in meat at retail (Ponnampalam, Trout et al. 2001). Management practices such as harvesting grass at different times of the growing season or allowing the grass to wilt before harvesting will influence the fatty acid proportions in the grass and thus in the meat of cattle and sheep (Wood, Richardson et al. 2007). Feed types will influence PUFA concentrations in meat due to levels of certain PUFAs or due to differences in feed processing in the rumen.

Despite pastures containing higher levels of PUFAs and antioxidants compared to concentrate diets, ruminants finished on pastures do not consistently produce meat with better colour and lipid stability. This may be due to differences in pasture vegetation

stage (varying in content of PUFA and antioxidant), concentrate diets (containing more linoleic acid, a PUFA) and animal breeds (Li and Liu 2012).

2.5.3.3 Age and maturity

The proportion of type I oxidative fibres in muscle increases with age (Warner, Pethick et al. 2007) and maturity (White, McGavin et al. 1978, Suzuki and Cassens 1983). Muscles from older more mature animals therefore have a more oxidative fibre type (Brandstetter, Picard et al. 1998, Greenwood, Harden et al. 2007), increased oxidative capacity (Gardner, Hopkins et al. 2007), and higher concentrations of myoglobin (Kelman, Pannier et al. 2014), iron and zinc (Pannier, Ponnampalam et al. 2010). Increasing lamb age is therefore associated with darker but redder bloomed meat colour and reduced colour stability on retail display. Jacob (2007) reported significantly worse colour stability in the loin muscle of 12 month old lambs compared to 5 month old lambs. The older lambs also had higher myoglobin concentrations. These results were consistent with those of Pethick et al. (2005) who reported that muscle myoglobin concentrations were higher in hoggets than lambs. Warner et al. (2007) found that lamb loins were darker, redder and yellower with a higher R630/R580 as lamb age increased from 8 to 14 to 22 months. Lambs are generally slaughtered at an older age in Australia (6 to 9 months or older) than in Europe, where much younger lambs produce a paler meat colour (Callejas-Cárdenas, Caro et al. 2014).

2.5.3.4 Growth

A range of production and genetic factors including lamb sex, maturity, breed and nutrition influence growth. Lamb maturity, breed and nutrition have been demonstrated to influence meat colour, however a direct association between lamb growth and meat colour has not been established. In Australia the use of sire breeding values for growth has increased lamb growth rates (Hall, Gilmour et al. 2002), resulting in lambs reaching

slaughter weight more rapidly and being less mature at slaughter (Hall 2000). Increasing lamb growth may therefore be associated with reduced muscle oxidative capacity (Brandstetter, Picard et al. 1998) and influence the display colour of lamb meat.

2.5.3.5 Breed

Animal genetics determined by their breed influences intrinsic muscle characteristics (Schreurs, Garcia et al. 2008) such as muscle oxidative capacity and thus influences meat colour. Lamb breeds differ in their muscling potential, their mature body weight and in the length of time they take to reach maturity (growth rate) (Schreurs, Garcia et al. 2008). Sire breed types are classified by Sheep Genetics in Australia as Terminal, Maternal or Merino (Brown, Huisman et al. 2007). Terminal breeds such as Suffolk, Texel and Poll Dorset are characterised by rapid lean growth of lambs, Maternal breeds such as Border Leicester and Dohne Merino have well-developed mothering and reproductive traits, while Merino breeds such as Poll Merinos are specialised for wool production.

Terminal and Maternal sires produce lambs with greater muscling potential and thus with greater lean meat yield. Increased muscling is associated with reduced muscle oxidative capacity (Greenwood, Gardner et al. 2006, Greenwood, Harden et al. 2007) due to an increased proportion of type IIX glycolytic muscle fibres (Wegner, Albrecht et al. 2000). Lamb breeds with greater muscling potential may therefore produce lighter, less red meat that has improved colour stability on retail display. In support of this thinking, Merino lambs have been reported to produce meat that browns faster on retail display (Warner, Kearney et al. 2017). Alternatively, the poor colour stability of Merino meat may relate to increased meat ultimate pH (Warner, Ponnampalam et al. 2007) or to Merino lambs being relatively older at the time of slaughter due to their slower growth and late maturation (Hopkins, Stanley et al. 2007).

2.5.3.6 Australian Sheep Breeding Values (ASBVs)

There has been an increasing shift towards meat production in the lamb industry with falling wool prices (Banks 2002, Curtis, Dolling et al. 2006). Consumers prefer large and lean lamb cuts with minimal fat, thus the Australian industry has focused on producing large, lean lamb with increased average slaughter weights (Hall, Gilmour et al. 2002, Pethick, Banks et al. 2006). Producers have successfully increased the lean meat yield of lamb carcasses through simultaneous selection for improved lamb growth, muscling and against fat depth (Banks, Ball et al. 2002) using sire Australian Sheep Breeding Values (ABSVs) for post-weaning weight (PWT), eye muscle depth (PEMD), and post-weaning c-site fat depth (PFAT) (Anderson, Pethick et al. 2016). Sire ASBVs are derived from measurements on the individual animals, the performance of their relatives, and appropriate genetic parameters (Fogarty 1995, Brown, Huisman et al. 2007) to provide an estimate of how a sire's progeny will perform for a particular trait (Brown, Ball et al. 2006). ASBVS are expressed as either positive or negative deviations from an average number and are calculated within Terminal, Maternal and Merino sire breed types.

While sire selection for increased PWT, PEMD and reduced PFAT has successfully increased lean meat yield, it has also had the unintended impact of reducing IMF content and thus the eating quality of lamb meat (Pannier, Pethick et al. 2014). Given their influence on muscle oxidative capacity and IMF, sire ASBVs for PEMD, PFAT and PWT are likely to influence the display colour of lamb meat. Meat colour traits have a genetic component for lamb (Mortimer, van der Werf et al. 2014) and beef (King, Shackelford et al. 2010), providing an opportunity for genetic selection to improve retail meat colour.

Post weaning eye muscle depth (PEMD)

Sire PEMD estimates are based on ultrasound measurement of loin muscle depth at the level of the 12th rib, adjusted for live weight (Hall, Gilmour et al. 2002). Lambs from high PEMD sires have higher lean meat yield due to increased muscling (Gardner, Williams et al. 2010). PEMD also causes some level of muscle redistribution to higher valued loin cuts, increasing the value of the carcass (Anderson, Williams et al. 2015). Selection for increased PEMD increases lamb loin muscle depth at the c-site (Hall, Gilmour et al. 2002, Hegarty, Hopkins et al. 2006, Hegarty, Shands et al. 2006) by ~ 0.61 mm for each mm increase in sire PEMD, irrespective of the plane of nutrition (Hegarty, Shands et al. 2006). The progeny of high PEMD sires have higher proportions of glycolytic type IIX muscle fibres (Greenwood, Gardner et al. 2006), reduced oxidative muscle fibres (Gardner, Pethick et al. 2006) and reduced myoglobin and iron concentrations (Pannier, Ponnampalam et al. 2010, Kelman, Pannier et al. 2014).

Post weaning fat depth (PFAT)

Sire PFAT estimates allow producers to select for reduced external carcass fat in lambs. PFAT estimates were developed using ultrasound measures of fat depth 45 mm from the mid-line over the 12th rib (the c-site) in live sires (Fogarty 1995, Hall, Gilmour et al. 2002, Hegarty, Shands et al. 2006), GR tissue depth (110 mm from the midline over the 12th rib) (Hegarty, Shands et al. 2006) and loin fat weight in lamb carcases (Gardner, Williams et al. 2010). Selection for reduced PFAT has been shown to increase lean meat yield in lambs (Gardner, Williams et al. 2010). PFAT and PEMD appear to impact most markedly at the site of their measurement (the c-site) (Gardner, Williams et al. 2010). In line with increasing lamb muscling, selection for reduced PFAT has been associated with reduced muscle oxidative capacity ICDH activity and myoglobin concentration in the loin muscle (Kelman, Pannier et al. 2014). Gardner et al. (2010)

found that PFAT had a greater influence on lean meat yield than PEMD or PWT, as selection for reduced PFAT decreased whole carcass fatness and increased muscularity (Gardner, Williams et al. 2010). Additionally, Anderson, Pethick and Gardner (2016) concluded that the greatest gains in lean value of lamb carcases were made by reducing PFAT, followed by increasing PEMD and PWT.

Post weaning weight (PWT)

Sire PWT estimates are based on lamb live weights corrected to 225 days of age. Selection for high PWT sires produces heavier lambs (Fogarty, Gilmour et al. 1997, Hall, Gilmour et al. 2002) that have an increased growth rate (Thompson, Parks et al. 1985), mature size (Huisman and Brown 2008) and carcass weight at slaughter (Gardner, Williams et al. 2010, Gardner, Williams et al. 2015). High PWT sires produce lambs that are faster growing due to their larger mature size, are relatively less mature when slaughtered at a set carcass weight (Huisman and Brown 2008) and that have relatively leaner carcasses with reduced muscle oxidative capacity (Brandstetter, Picard et al. 1998). Selection for high PWT sires may thereby influence meat colour. Hopkins, Hegarty and Farrell (2005) reported a positive association between PWT and lamb meat *L**, however further work by Hopkins et al. (2007) found no effect of sire PWT estimates on meat colour. Gardner et al. (2010) found that PWT altered carcass composition by reducing fatness and increasing bone, but changed muscle content very little. Therefore the extent that selection for high PWT sires influences meat colour remains unclear.

2.5.3.7 Sex

While meat quality traits are influenced by lamb sex (Butler-Hogg, Francombe et al. 1984, Dransfield, Nute et al. 1990, Horcada, Beriain et al. 1998, Greenwood, Harden et al. 2007) there is little evidence that lamb sex influences meat colour (Hopkins and

Fogarty 1998, Horcada, Beriain et al. 1998, Teixeira, Batista et al. 2005, Tejeda, Peña et al. 2008). Greenwood et al. (2007) reported that female lambs had a higher proportion of glycolytic type IIX muscle fibres, however this change did not influence meat colour.

2.6 General Aims and Hypotheses

The aim of this study was to describe the phenotypic and genotypic factors influencing the colour of lamb meat on retail display. Thousands of mixed breed lambs of known genetics were produced over 5 years across Australia as part of the Sheep CRC's INF experiment. The lambs were measured for a multitude of traits from birth until slaughter, when they were measured for a wide range of carcass and muscle traits including bloomed meat colour and its stability over a 3 day simulated retail display. This experiment thereby facilitates a comprehensive analysis of the influence that Australian production factors, carcass and muscle traits and sire ABVS have on lamb meat colour on retail display.

The different aspects of this study are covered in the following chapters; the hypotheses of which are outlined below:

Chapter 3: Production factors influence fresh lamb *longissimus* colour more than muscle traits such as myoglobin concentration and pH.

The aim of this chapter was to quantify and clarify the muscle traits of importance in determining the colour of freshly bloomed lamb meat produced under commercial conditions; to compare these effects with those of different production factors and to determine if the production factors may be accounted for by changes in muscle traits.

The specific hypotheses tested were:

- Muscle pH would have a greater effect on lamb loin lightness than myoglobin and iron concentration or ICDH activity, a marker of muscle oxidative capacity (Gardner, Hopkins et al. 2007).

- Muscle factors such as pH, myoglobin and iron concentration would be more important determinants of fresh lamb lightness than production factors including the site and year of production, slaughter group, sex, breed and age of the lamb.

- Myoglobin and total iron concentration and ICDH will have a greater effect on lamb meat redness, yellowness, hue angle and chroma than other intrinsic muscle factors and all production factors measured in this study.

Chapter 4: Selection for intramuscular fat and lean meat yield will improve the bloomed colour of Australian lamb loin meat.

The aim of this chapter was to assess the associations between IMF, genetic and phenotypic indicators of lean meat yield and fresh lamb loin colour parameters, reporting the magnitudes of impact that selection for increased IMF and lean meat yield will have on these parameters.

The specific hypotheses tested were:

- The progeny of high PWT, high PEMD and low PFAT sires would produce lamb loins with increased L^*, b^* and hue angle values, and reduced a^* and chroma values due to reduced muscle oxidative capacity.

- Reducing IMF concentration in lamb loins would increase surface L^*, b^* and hue angle, and reduce a^* and chroma.

Chapter 5: Factors affecting the colour of lamb meat from the *longissimus* muscle during display: The influence of muscle weight and muscle oxidative capacity.

The aim of this chapter was to assess the association between R630/R580 measures in the *longissimus lumborum* muscle after 3 days of simulated retail display with phenotypic carcass measurements relating to lean meat yield, ICDH activity and sire ASBVs for muscling and growth.

The specific hypotheses tested were:

- Loin meat derived from lambs with heavier muscle weight would be redder in colour (higher R630/R580) after 3 days of display than meat with high ICDH activity.
- Lamb meat from the progeny of high PEMD sires would have increased R630/R580 on display.
- Meat from rapidly growing lambs, and lambs from high PWWT sires would also have increased R630/R580 at the end of display.

Chapter 6: Initial bloom colour is a poor predictor of colour stability in lamb *longissimus* meat

The aim of this chapter was to determine whether meat redness (R630/R580) measured at the start of display may be used in conjunction with a lamb's production and/or carcass information to provide an accurate prediction of the meat browning after 3 days of retail display.

The specific hypothesis tested was:

- Knowledge of lamb age, loin meat pH_{24}, IMF concentration and surface R630/R580 at the start of retail display would allow the prediction of lamb loin redness (R630/R580) at the end of a 72 hour simulated retail display period

Chapter 7: Dietary vitamin E supplementation reduces lamb meat browning on display following up to 70 days of chilled storage.

The aim of this chapter was to examine the ability of dietary Vitamin E supplementation to improve the colour stability in long-stored lamb meat with high IMF, ICDH activity and pH, given the likely increased oxidative load triggering browning in this meat.

The specific hypotheses tested were:

- Vitamin E supplementation would reduce the browning of lamb loin on retail display to a greater extent in long-stored meat.
- The effect of vitamin E supplementation on display colour would increase in lamb meat with high IMF, ICDH activity and pH.

Chapter 3. Production factors influence fresh lamb *longissimus* colour more than muscle traits such as myoglobin concentration and pH

This chapter is published in Meat Science:

Calnan, H.C., Jacob, R.H., Pethick, D.W. and Gardner, G.E. (2016). Production factors influence fresh lamb *longissimus* colour more than muscle traits such as myoglobin concentration and pH. Meat Science. 119: 41-50.

Abstract

M.*longissimus* colour was measured from 8165 lambs at 24 hours *post-mortem* using a chromameter. The impact of production factors (site and year of production, slaughter group, sex, age and breed type) and muscle traits (hot carcass weight, pH_{24}, isocitrate dehydrogenase (ICDH) activity, myoglobin, iron and zinc concentrations) on meat lightness (*L**), redness (*a**), yellowness (*b**), hue and chroma were analysed. Greater differences in meat colour were seen between different slaughter groups and sites of production than across the range of any muscle traits. Of the muscle traits analysed, changes in pH_{24} had the greatest effect on meat *a** (2.5 units), while myoglobin had the greatest effect on meat *L** (2.9 units). The 3.1 *L** unit darkening of meat with increasing lamb age (from 140 to 400 days) was accounted for by increased myoglobin concentration. These results suggest that production factors are having substantial effects on lamb colour independent of known influencing muscle traits such as myoglobin concentration and pH.

3.1 Introduction

The colour developed in fresh lamb meat following oxygenation is critical to the display colour of the meat and thus the purchasing decision of consumers. Consumers expect and demand that lamb meat has a bright-red colour and any discolouration will discourage their purchase. For this reason discoloured lamb meat is discounted, downgraded to a lower-value product such as mince, or discarded depending on the extent of the discolouration. This represents a substantial financial loss and reduction in supply chain efficiency for the lamb meat industry.

While the factors influencing the browning of red meat on display, or meat colour stability, has been the focus of the majority of research in red meat colour, the initial colour developed in lamb meat following exposure to oxygen is equally important in terms of consumer approval and has been far less extensively researched. The initial oxygenated colour of meat may be measured in carcasses 24 hours *post-mortem* after a muscle surface has been sliced and allowed to bloom for at least 30 minutes. Blooming is the dramatic colour change that occurs upon exposure of a meat surface to oxygen. As oxygen diffuses into a meat surface it binds with purple deoxymyoglobin to form red oxymyoglobin- the pigment primarily responsible for red meat's colour (AMSA 2012). The extent of meat blooming determines meat colour development and depends on both the depth of oxygen penetration and the proportions of deoxymyoglobin and oxymyoglobin pigments within a meat surface. A reduction in bloom depth or oxymyoglobin formation will lead to meat that appears too dark, pale and/or dull in colour and is thus likely be rejected by consumers (MacDougall 1982).

A number of production factors, intrinsic muscle factors and external *post-mortem* factors influence the depth of oxygen penetration into meat, its binding to myoglobin and thus the colour of fresh lamb meat. Important *post-mortem* factors impacting meat

colour include the ambient oxygen concentration, its duration of exposure to the meat surface and the ambient temperature (AMSA 2012). The strong influence of temperature on meat colour has been well established and is thus usually tightly controlled by lamb processors to optimise red meat colour (AMSA 2012). In contrast to these clearly established external influences on meat colour, the relative importance of different lamb production factors and intrinsic muscle traits in determining lamb meat colour remains unclear.

Certain production factors such as lamb breed, slaughter weight and nutrition influence fresh lamb meat colour (Hopkins and Fogarty 1998, Priolo, Micol et al. 2002, Martínez-Cerezo, Sañudo et al. 2005, Teixeira, Batista et al. 2005, Ripoll, Joy et al. 2008). Hopkins and Fogarty (1998) reported that colorimetric measures of meat *L**, *a** and *b** differed by up to 1.6, 1.1 and 0.9 units between lamb breeds, while Teixeira et al. (2005) reported smaller differences of 0.3, 0.6 and 0.7 units. Teixiera et al. (2005) also reported that lamb live weight within an age range of 90 - 120 days had a large effect on *L** and *b** (5.0, 6.1) though not on *a** (1.1), while only small differences in meat *L**, *a** and *b** (1.5, 0.8 and 0.3) were measured between the sexes. Priolo et al. (2002) showed that different feeding systems had a moderate effect on *L** (3.1) though little on *a**, *b** or Chroma (0.2, 0.9, 0.6), while growth rates had negligible effect on all parameters (0.4, 0.3, 0.04, 0.2). Ripoll (2008) reported different housing and feeding production systems changed mean meat *L**, *a**, *b** and chroma by 2.0, 2.1, 1.3 and 2.3 units. Dietary Vitamin E intake influences the fresh colour of lamb meat (Pearce, Masters et al. 2005) in addition to delaying browning during meat display (Jose, Pethick et al. 2008), however these studies reported the effects of vitamin E using spectrophotometric measures that are not directly comparable with colorimetric measures.

The mechanisms through which production factors impact fresh lamb colour has not been clearly established, though may in part be associated with changes in intrinsic muscle factors such as pH, myoglobin content or oxidative capacity; all of which influence the oxidation of myoglobin. A high ultimate pH is known to cause darkening of meat (Lawrie 1983, Faustman and Cassens 1990) and is anecdotally considered by industry to be the most important cause of dark red meat. High muscle myoglobin and iron concentrations reduce meat lightness and increase the redness and vividness of meat colour (Ledward 1992), while muscle's oxidative capacity influences meat colour as more oxidative fibre types contain higher myoglobin and iron concentrations (Suzuki and Cassens 1983) and metabolise more oxygen *post-mortem* reducing oxymyoglobin formation. The magnitude of effect of muscle oxidative capacity, myoglobin, iron and pH on lamb meat colour relative to other intrinsic muscle factors and to production factors is unclear, as is the extent that these muscle factors underpin production factor influences. Improved understanding of these interacting factors and their relative magnitudes of effect in determining lamb meat colour is needed in order for the lamb meat industry to reduce product variability and waste caused by unappealing colour development in lamb.

This research aims to quantify and clarify the muscle traits of importance in determining the colour of fresh lamb meat produced under commercial conditions; to compare these effects with those of different production factors and to determine if the production factors may be accounted for by changes in muscle traits. We present three hypotheses: 1. Muscle pH will have a greater effect on lamb loin lightness than myoglobin and iron concentration or isocitrate dehydrogenase (ICDH) activity, a marker of muscle oxidative capacity (Gardner, Hopkins et al. 2007). 2. Muscle factors such as pH, myoglobin and iron concentration will be more important determinants of fresh lamb lightness than production factors including the site and year of production, slaughter

group, sex, breed and age of the lamb. 3. Myoglobin and total iron concentration and ICDH activity will have a greater effect on lamb meat redness, yellowness, hue and chroma than other intrinsic muscle factors and all production factors measured in this study.

3.2 Materials and Methods

3.2.1 Animal, genetic and production details

Data were collected from 8165 lambs produced in the Sheep Cooperative Research Centre Information Nucleus Flock (INF) over a 5 year period (2007 - 2011) at 8 sites across Australia (AEC numbers 1-7-02, 1-10-1 and 3-10-13), and has been comprehensively described previously (Fogarty, Banks et al. 2007, van de Werf, Kinghorn et al. 2010). Fresh colour was measured from lambs reared at each of the eight sites; Cowra, Kirby and Trangie in New South Wales, Hamilton and Rutherglen in Victoria, Struan and Turretfield in South Australia, and Katanning in Western Australia (Table 3-1).

The lambs were the progeny of 474 different sires, with approximately 90 sires used each year across all sites. These sires comprised Terminal sire types (Hampshire Down, Ile De France, Poll Dorset, Southdown, Suffolk, Texel and White Suffolk), Maternal sire types (Bond, Booroola, Border Leicester, Coopworth, Corriedale, Dohne Merino, East Friesian, Prime SAMM and White Dorper) and Merino sire types (Merino and Poll Merino). Semen from all three sire types was used to artificially inseminate Merino dams, while only semen from Terminal sires was used to inseminate cross bred ewes (eg Border Leicester x Merino dams). Hence the lambs were of four sire type and dam breed combinations; Maternal ram and Merino ewe, Merino ram and Merino ewe, Terminal ram and Merino ewe or Terminal ram and Merino cross bred ewe (Table 3-1).

Maternal and Merino sired lambs sent to slaughter comprised very few females (which were retained for breeding purposes), meaning effective comparisons between sexes could only be made within the Terminal sired lamb groups.

The lambs were maintained on extensive pasture grazing, with grain, hay or feedlot pellets supplemented when pasture supply was limited. Further details of breed types used, lamb feeding and management are described elsewhere (van de Werf, Kinghorn et al. 2010, Ponnampalam, Butler et al. 2012, Ponnampalam, Butler et al. 2014).

Table 3-1 Number of lambs measured for *m. longissimus lumborum* colour at each site, within each year, sex, dam breed & sire type

Site	Year of Birth					Sex Dam breed (Sire type)					
	2007	2008	2009	2010	2011	M Mer. (Mat.)	M Mer. (Mer.)	M Mer. (Ter.)	F Mer. (Ter.)	M Mer-x (Ter.)	F Mer-x (Ter.)
Cowra 1555	228	392	378	365	192	311	246	183	202	317	296
Kirby 743	-	218	199	203	123	135	133	89	95	143	148
Trangie 1086	283	149	199	196	259	194	162	200	163	203	164
Hamilton 1115	291	213	208	207	196	177	148	96	74	316	304
Rutherglen 753	197	193	175	188	-	132	113	146	164	106	92
Struan 733	275	122	172	164	-	137	74	55	50	214	203
Turretfield 939	262	221	214	242	-	187	156	201	193	94	108
Katanning 1241	162	278	23	382	396	284	258	225	212	125	137

M: Male, F: Female, Mer.: Merino, Mer-x : Cross-bred Merino, Mat. : Maternal, Ter. : Terminal.

3.2.2 Slaughter details

Data were collected At each site, lamb weight was used to create smaller groups of lambs to send to the abattoir for slaughter on the same day (slaughter groups) with target average carcass weight of 20- 25 kg. The lambs from 8 sites were slaughtered at 5 different abattoirs. There were a total of 125 slaughter groups in this study, that contained an average of 65 lambs in each, though the number of lambs within slaughter groups ranged from 23 to 128 depending on the number of lambs that achieved target

carcass weight. Given that selection for slaughter was made based on weights, the lambs ranged from 134 to 504 days of age at the time of slaughter. Lambs were born in clusters following artificial insemination thus the average age range within slaughter groups was only 11 days.

The day prior to slaughter, slaughter groups of lambs were removed from their paddocks and held in a pen for 6 hours before weighing and transportation to a commercial abattoir. Trucking distances between the production sites and abattoirs varied considerably, though were consistent for each site in this study. The lambs were held in lairage overnight at the abattoir before slaughter the following morning. All carcasses were subjected to medium voltage electrical stimulation (Pearce, van de Ven et al. 2010) and trimmed according to AUS-MEAT specifications (Anonymous 2005).

3.2.3 Carcass and muscle sampling details

The hot carcass weight (HCWT, kg) of each lamb was measured on the chain following slaughter. A small portion of loin muscle was collected adjacent to the 12th rib as soon as possible (a maximum of 5 hours) *post-mortem* to measure ICDH enzyme activity. A 1 g portion of muscle was snap-frozen in liquid nitrogen and stored at -80 °C until ICDH activity could be measured according to the method of Briand (1981).

All carcasses within a slaughter group were hung in a chiller at 3 - 4 °C for between 20 to 25 hours before the *m. longissimus thoracis* (caudal from 12th rib region) *et lumborum* (to the lumbar sacral junction) or loin muscle was removed from each carcass. A TPS WP-80 pH and temperature meter (with a Mettler Toledo puncture pH probe- LoT406-M6-DXK-S7/25) was calibrated at pH 4 and 7 within the chiller and then used to measure loin muscle temperature and pH (pH_{24}) via the insertion of probes into the centre of the loin muscle adjacent to the 12th rib, as further described by Pearce

et al. (2010). The carcasses had an average muscle temperature of 3.6 °C (±1.9) at this time.

From the excised muscle small portions were sampled from the posterior section and frozen for subsequent measurement of myoglobin, total iron and zinc concentrations. For myoglobin concentration, a 1g sample of loin muscle was excised, finely diced and stored at -20 °C in a 5ml tube until analysis. Approximately 0.2 g of muscle tissue was then homogenised in 0.04 M phosphate buffer (pH 6.5) using a Polytron PT 10-35 (Kinematica, Lucerne, Switzerland) at 30,000 rpm for 20 seconds. Samples were then centrifuged for 10 minutes at 300 rpm and the supernatant collected. The supernatant was incubated at room temperature for 60 minutes before Trition X-100 (10 %) and 65 mM sodium nitrite were added to the supernatant. The myoglobin assay was then performed using a Beckman DU650 spectrophotometer, with absorbance read at 730 (turbidity) and 409 nm (oxidised pigment) before myoglobin concentration was estimated using the method of Trout (1991).

Loin samples were frozen at –20 °C before freeze-drying using a Cuddon FD 1015 freeze dryer (Cuddon Freeze Dry, Blenheim, New Zealand) for the determination of total iron and zinc concentrations. Approximately 0.2 g dry matter per sample was weighed and prepared according to the USEPA method 200.3 (USEPA 1991). Iron and zinc concentrations were then determined using a Vista AX CCD simultaneous ICP-AES (Varian Australia Pty Ltd).

3.2.4 Meat colour measurement

The *m. longissimus lumborum* of each lamb was sliced perpendicular to the long axis of the muscle and adjacent to the 12th rib at approximately 24 hours *post-mortem*. The cut surface was exposed to air within a 3 – 4 ° C chiller for 30 - 40 minutes to allow

blooming prior to colour measurement. The bloomed surface colour of the meat was then measured using a Minolta Chromameter, Model CR – 400 (Konica Minolta Optics, Inc.), with a different machine used at each abattoir.

The Minolta chromameter was fitted with a closed cone, set on a "D65" illuminant, using a 2° standard observer and 10 mm measurement area (aperture). The chromameter was fitted with a CR-A33a or CR-A33f light projection tube with a glass 'shield' fitted to the base of the measuring head and was calibrated prior to use using a white tile (according to manufacturer's instructions) within the chiller where measurements were subsequently taken.

The chromameter measured colour according to the Commission International de l'Eclairage (CIE) $L^*a^*b^*$ system (CIE 1976); where L^* or lightness represent light to dark on a scale of 100 to 0; a^* or redness represents red to green on a scale of +60 to -60; and b^* or yellowness represents yellow to blue on a scale of +60 to -60. Hue angle was calculated manually (arctangent(b^*/a^*)) with values from 0-360° representing a colour based on a colour circle, where 0 (=360) represents red, 90 is yellow, 180 is green and 270 is blue. Thus in the context of meat colour, hue values closest to 0 or 360 are considered optimal. To correct for negative b^* values creating negative hue angles, 360 was added to all hue values to allow analysis, before being subtracted from any values exceeding 360, according to the method of McGuire (1992). Chroma or the colour saturation index was manually calculated as ($\sqrt{(a^{*2} + b^{*2})}$), where higher values represent more vivid and thus optimal meat colour.

Three replicate measurements of L^*, a^* and b^* were taken of the bloomed meat surface before averaging for analysis. The chromameter head was rotated by 45 ° for each measure and effort was made by the operator to avoid areas of dense connective tissue or IMF.

3.2.5 Statistical Analysis

Due to the fundamental associations that exist between *L**, *a** and *b** within the visible light spectra, these variables were initially analysed using a multivariate regression analysis (SAS Version 9.1, SAS Institute, Cary, NC, USA). In this multivariate regression, *L**, *a** and *b** were fitted as dependent variables, while fixed effects for site, year of birth, slaughter group (within site by year of birth), sex and dam breed within sire breed type, as well as all relevant first order interactions between these terms were tested. Non-significant ($P > 0.05$) terms were removed in a step-wise fashion with remaining significant effects ($P < 0.05$) forming the fixed base model for further analysis of *L**, *a** and *b** (Table 3-2). This base model was used to analyse *L**, *a** and *b** in separate linear mixed effects models, where sire identification and dam identification by year of birth could be included as random terms.

The next phase of the analysis involved testing the associations between *L**, *a** and *b** and covariate muscle effects (HCWT, pH_{24}, myoglobin, iron, ICDH activity and zinc concentration). These associations were first tested in a multivariate regression, where covariates were incorporated one at a time into the base model described above (Table 3-2) for *L**, *a** and *b** together. Each model included one covariate along with all its relevant first order interactions with fixed effects and squared covariate terms to test for curvilinear effects. Non-significant ($P > 0.05$) terms were removed in a stepwise manner to form the base model for each covariate. As described previously, the model defined through this multivariate regression was then applied to the individual analysis of *L**, *a** and *b** in separate linear mixed effects models, with random terms for sire identification and dam identification by year of birth. This process was then repeated with each covariate also fitted with HCWT incorporated into the model, to ensure that any observed associations were not simply reflecting changes in HCWT.

Hue angle and chroma were analysed individually using linear mixed effects models. All fixed effects (site, year, slaughter group within site and year, sex and dam breed within sire breed type) and their first order interactions were included in the initial model, along with random terms for sire identification and dam identification by year of birth, with non-significant ($P > 0.05$) terms removed in a step-wise fashion to form the base models described in Table 3-2. In a second phase of this analysis, each covariate (HCWT, pH_{24}, myoglobin, iron, ICDH activity and zinc concentration) was then incorporated into the base model one at a time along with their first order interactions with fixed effects and squared terms. Non-significant ($P > 0.05$) terms were removed in a stepwise manner to form each covariate model. This process was repeated with HCWT included with each covariate in the model.

The same approaches described above were taken when testing the impact of lamb age at slaughter on *L**, *a**, *b**, hue angle and chroma, with a few key differences. Given the limited age range within each slaughter group (average range of 11 days), when lamb age was included in the model as a covariate the slaughter group within site by year term was removed as a fixed effect and instead fitted as an additional random term. This was done for all linear mixed effects models testing an association with lamb age.

The magnitude of each fixed effect on lamb loin colour measures are expressed as the unit difference between minimum and maximum predicted means for *L**, *a**, *b**, hue angle and chroma between different fixed production factors (Figure 3-1). The magnitude of covariate effects on *L**, *a**, *b**, hue angle and chroma are expressed as the unit change in predicted *L**, *a**, *b** hue angle and chroma across the range of each carcass or muscle covariate (Figure 3-1).

Table 3-2 F-values, *P*-values and numerator and denominator degrees of freedom (NDF, DDF) for the base linear mixed effects models for *L**, *a**, *b**, hue angle and chroma of the *m. longissimus lumborum* of lambs.

Effect	NDF, DDF	F-values				
		*L**	*a**	*b**	Hue angle	Chroma
Site	7, 1424	507	1047	8933	14705	541
Year	4, 6141	102	227	62	122	230
Slaughter Group (site x year)	113, 1424	46	71	229	292	67
Sex | Dam breed (sire type)	5, 1424	13	6	10	12	6

All effects have a *P* value < 0.001.

3.3 Results

The number, mean, standard deviation and range for the fresh colour data and all muscle covariates tested are shown in Table 3-3.

Table 3-3 Description of dependent variable and covariate data

	Number	Mean	St. Dev.	Range
*L**	8165	34.9	3.07	24.4 – 46.0^
*a**	8165	18.4	2.35	9.8 – 30.0^
*b**	8165	3.8	4.13	-5.4 – 19.3^
Hue angle	8165	11.3	12.52	-18.6 – 40.3^ #
Chroma	8165	19.3	2.36	10.1 – 32.2^
Covariates:				
HCWT (kg)	8074	23.1	3.72	12.5 – 40.0
pH_{24}	8149	5.7	0.14	5.2 – 6.9
Myoglobin (mg/g muscle)	5032	6.6	1.88	2.2 – 15.6
Iron (mg/100g muscle)	5016	20.4	3.66	8.1 – 45.1
ICDH activity (µmol/min/g)	2881	5.0	1.61	1.0 – 11.3
Zinc (mg/100g muscle)	5016	24.4	4.39	12.0 – 44.9
Age (days)	8163	260.0	71.8	134 – 504

^Scales of *L**, *a**, *b**, Hue angle & Chroma: *L**: -100 to 100, *a** & *b**: -60 to 60, Hue angle: 0-360°, Chroma: 0-60

Hue angles between 340-360° have been displayed as negative values (= minus 360) to show a continuous unit range (ie 341.4° depicted as -18.6)

3.3.1 Effect of production factors on *L**, *a**, *b**, Hue angle and Chroma– base model

The mean instrumental colour measures for all lambs (n= 8165) (± SD) produced over five years was 34.9 (± 3.07) units for *L**, 18.5 (± 2.33) for *a** and 3.8 (± 4.13) for *b** (Table 3-3). The base models outlined in Table 2 describe 65%, 71% and 94% of the variance in *L**, *a** and *b** respectively. The *b** values within this data set were strongly correlated with *L** and *a** (*L** vs *b** = 0.4; *a** vs *b** = 0.6), although there was no correlation between *L** vs *a** (0). A mean hue angle of 11.3 (± 12.5) and chroma of 19.3 (± 2.36) were calculated using *a** and *b** values. The base models for hue angle and chroma (Table 3-2) described 96% and 66% of the variance in these measures.

The magnitude difference between the minimum and maximum mean *L**, *a**, *b**, hue angle and chroma values between different fixed production effects are shown in Figure 1a. Different sites of lamb production differed markedly in loin *L**, *a** and particularly *b** ($P < 0.01$; Table 3-4, Figure 3-1), with these differences varying each year, while the change in these colour measures between years was considerably less (Figure 3-1). *L** values varied by up to 4.2 units between different sites of production and by only 2.4 units between years, while *a** differed by up to 3.7 units between sites and by only 1.3 units between years (Table 3-4, Figure 3-1). Up to 8.4 units difference was measured in *b** values at different sites of production, compared to only 1.6 units difference between years of production (Table 3-4, Figure 3-1). Hue angle also varied substantially more (by up to 26.9 units) between different sites of production than between years of production (5.0 units), while chroma differed by up to 2.6 units between sites and by up to 1.5 units between years (Table 3-4, Figure 3-1).

The greatest difference in mean *L**, *a**, hue angle and chroma values was seen between different slaughter groups of lambs, compared within the same site and year of

production ($P < 0.01$; Figure 3-1), though no consistent trend was demonstrated across sites or years. Within any site of production in any one year, the mean slaughter group loin L^* varied by up to 6.5 units, a^* by up to 5.7 units, hue angle by up to 22.5 units and chroma by up to 5.7 units difference (Table 3-4, Figure 3-1). Alternatively there was less difference in loin b^* values observed between slaughter groups (7.9 units) within any one site and year, than was measured between sites of production (8.4 units) (Figure 3-1). The minimum and maximum predicted means for L^*, a^*, b^*, hue angle and chroma from different production sites, years, and between different slaughter groups (at any one site and year) are shown in Table 4. The unit difference between these minimum and maximum predicted means for L^*, a^*, b^*, hue angle and chroma for each fixed production effect is shown in Table 3-4 and Figure 3-1.

Comparisons between sire types can be made between male progeny from Merino dams. Terminal sired lambs produced the lightest coloured loin meat ($P < 0.01$) with a mean L^* value 0.3 units higher than Maternal and Merino sired lambs (Table 3-5; Figure 3-1). Maternal sires produced lamb loins with 0.2 unit higher mean a^* values than Merino and Terminal sired lambs as well as 0.1and 0.2 unit higher b^* values than Terminal and Merino sired lambs (Table 3-5; Figure 3-1). Maternal sired lambs thus also produced meat with the highest mean hue angle and chroma ($P < 0.01$). Maternal sired lamb loins had a mean hue angle 0.4 units higher than Merino lambs and 0.1 units higher than Terminal sired lambs, while the chroma of the Maternal sired lambs was only marginally (0.1 unit) higher than Merino and Terminal sired lambs (Table 3-5; Figure 3-1).

The effect of dam breed or of sex can only be compared among the progeny of terminal sired lambs. On average, the progeny of crossbred (Border Leicester–Merino) dams produced meat with 0.2 unit higher a^*, b^* and chroma and 0.6 unit higher hue angles

than progeny from Merino dams ($P < 0.01$; Figure 3-1), though no difference in mean loin L^* was evident ($P > 0.05$). On average male lambs produced loin meat with a 0.43 unit higher mean L^* than female lambs ($P < 0.01$; Figure 3-1), and while no significant differences in mean loin a^*, b^* or hue angle were shown between lamb sexes, female lambs produced loin meat with a 0.15 unit higher chroma than male lambs ($P < 0.01$; Figure 3-1).

Table 3-4 The minimum (Min), maximum (Max) and difference between (Diff) predicted means for lamb loin *L**, *a**, *b**, hue angle and chroma (± standard error) at different sites, years and between slaughter groups within any given site and year.

	*L**			*a**			*b**			Hue angle			Chroma		
	Min	Max	Diff	Min	Max	Diff	Min	Max	Diff	Min	Max	Diff	Min	Max	I
Site	33.2	37.4	4.2	16.2	19.9	3.7	-0.2	8.2	8.4	359.5	26.4	26.9	18.1	20.7	
	(0.15)	(0.13)		(0.10)	(0.12)		(0.10)	(0.08)		(0.24)	(0.21)		(0.12)	(0.14)	
Year	33.5	35.9	2.4	17.4	18.7	1.3	3.0	4.6	1.6	9.1	14.1	5.0	18.1	19.6	
	(0.15)	(0.15)		(0.10)	(0.10)		(0.09)	(0.08)		(0.21)	(0.20)		(0.11)	(0.14)	
Slaughter group	30.1	36.6	6.5	14.6	20.4	5.8	-3.1	4.8	7.9	349.5	12.0	22.5	14.7	20.4	
(site x year)	(0.25)	(0.24)		(0.22)	(0.17)		(0.13)	(0.16)		(0.34)	(0.33)		(0.19)	(0.24)	

Table 3-5 The predicted mean lamb loin *L**, *a**, *b**, hue angle and chroma (± standard error) for each sex and dam breed within sire type grouping.

Sex Dam breed (Sire type)	*L**	*a**	*b**	Hue angle	Chroma
M Merino (Maternal)	34.6 ^{ab}	18.4 ^{abc}	3.8 ^{b}	11.4 ^{a}	19.2 ^{abc}
	(0.07)	(0.04)	(0.04)	(0.09)	(0.04)
M Merino (Merino)	33.5 ^{ab}	18.3 ^{ab}	3.7 ^{a}	11.1 ^{a}	19.1 ^{ab}
	(0.09)	(0.06)	(0.05)	(0.12)	(0.06)
M Merino (Terminal)	34.9 ^{c}	18.2 ^{a}	3.7 ^{ab}	11.3 ^{a}	19.1 ^{a}
	(0.08)	(0.05)	(0.04)	(0.10)	(0.05)
M Merino Cross (Terminal)	34.8 ^{bc}	18.4 ^{bc}	3.9 ^{c}	11.9 ^{b}	19.3 ^{bc}
	(0.07)	(0.04)	(0.04)	(0.09)	(0.05)
F Merino (Terminal)	34.4 ^{a}	18.3 ^{ab}	3.8 ^{ab}	11.3 ^{a}	19.2 ^{ab}
	(0.07)	(0.05)	(0.04)	(0.10)	(0.05)
F Merino Cross (Terminal)	34.4 ^{a}	18.5 ^{c}	4.0 ^{c}	12.0 ^{b}	19.4 ^{c}
	(0.07)	(0.04)	(0.04)	(0.09)	(0.05)

M: Male, F: Female. Values in the same column followed by the same letter are not significantly from each other ($P \geq 0.05$)

	*L**	*a**	*b**	Hue	Chroma
a) Fixed Effects : Maximum unit difference in L*, a*, b*, hue and chroma between fixed effects					
Site of production	4.2	3.7	8.4	26.9	2.6
Year of birth	2.4	1.3	1.6	5.0	1.5
Slaughter group	6.5	5.8	7.9	22.5	5.7
Sire type	0.3	0.2	0.2	0.4	0.2
Dam breed	0.03$^{n.s.}$	0.2	0.2	0.6	0.2
Sex	0.4	0.1	0.04$^{n.s.}$	0.03$^{n.s.}$	0.1
b) Covariate Effects : Associations & unit effect on L*, a*, b*, hue and chroma across covariate ranges					

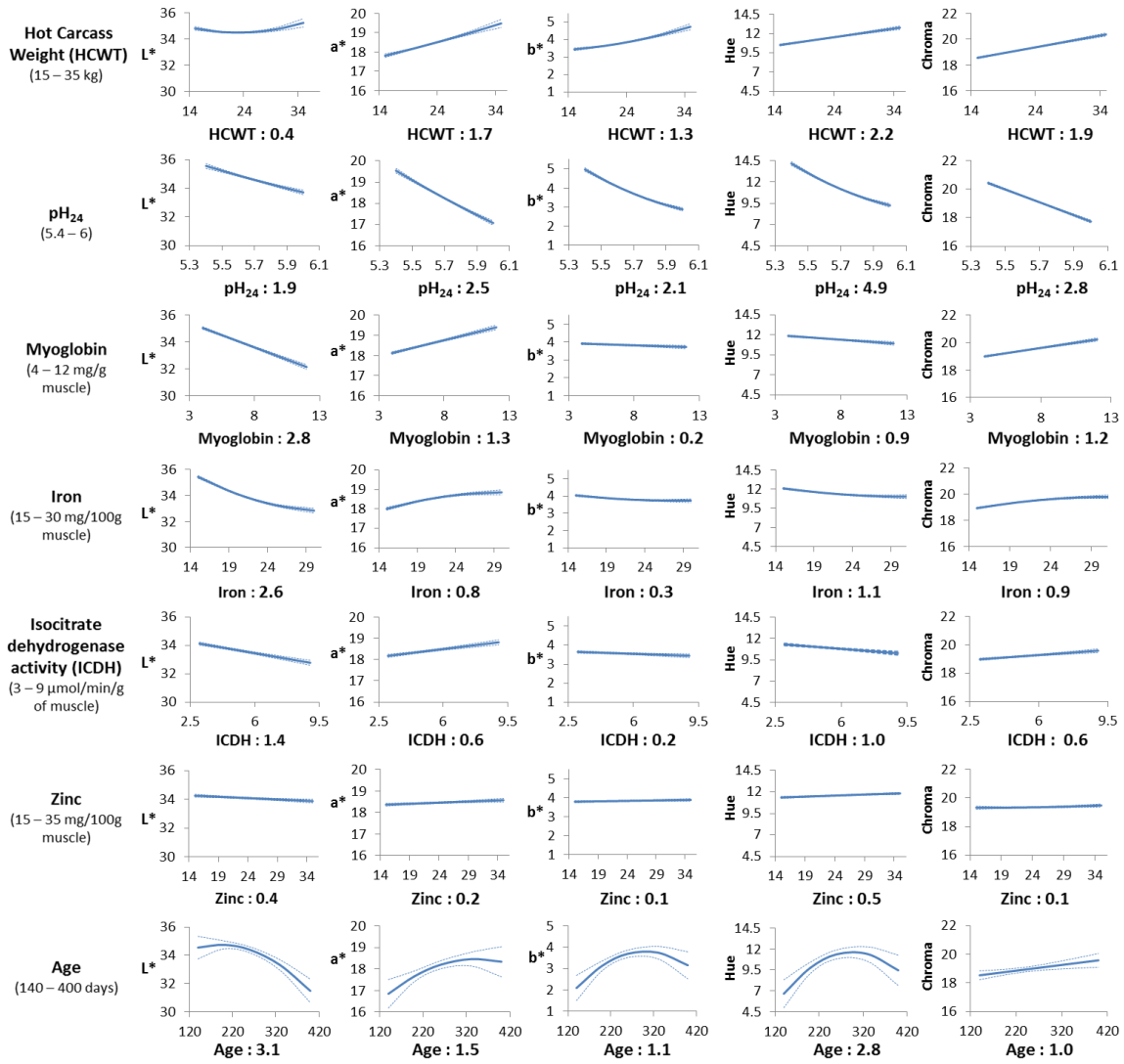

Figure 3-1 The impact of production factors and carcass traits on lamb loin *L**, *a**, *b**, hue angle and chroma.
a) Difference between the minimum and maximum mean lamb loin *L** (lightness), *a** (redness), *b** (yellowness), hue angle and chroma measured between different production effects (sites and years of production, slaughter groups within site and year, sire types, dam breeds and sexes within sire type). b) Figures depict the predicted association between *L**, *a**, *b**, hue angle and chroma and covariate effects: HCWT, pH_{24}, myoglobin, iron, ICDH, zinc and lamb age. Solid lines within figure represent predicted means while dotted lines represent the standard error of the mean. The number beneath each figure represents the unit change in *L**, *a**, *b**, hue angle and chroma across the listed range in each covariate.

3.3.2 Effect of carcass traits on *L**, *a**, *b**, hue angle and chroma

HCWT had a strong positive association with lamb loin *a**, *b**, hue angle and chroma ($P < 0.01$, Figure 3-1). With increasing HCWT from 15 to 35 kg there was an associated 1.7 unit increase in *a**, 1.3 unit increase in *b**, 2.2 unit increase in hue angle and 1.9 unit increase in chroma (Figure 3-1). Alternatively, loin *L** changed relatively little across this change in lamb HCWT, with the lowest *L** values predicted at approximately 24 kg HCWT and increasing by only 0.4 units in lambs with a 35 kg HCWT (Figure 3-1). Increasing pH_{24} of the loin muscle produced a marked and consistent decrease in mean *L**, *a**, *b**, hue angle and chroma values ($P < 0.01$, Figure 3-1). Increasing pH_{24} from 5.4 to 6 reduced lamb loin *L** by 1.9 units (Figure 3-2), *a** by 2.5 units (Figure 3-3), *b** by 2.1 units, hue angle by 4.9 units and chroma by 2.8 units (Figure 3-1).

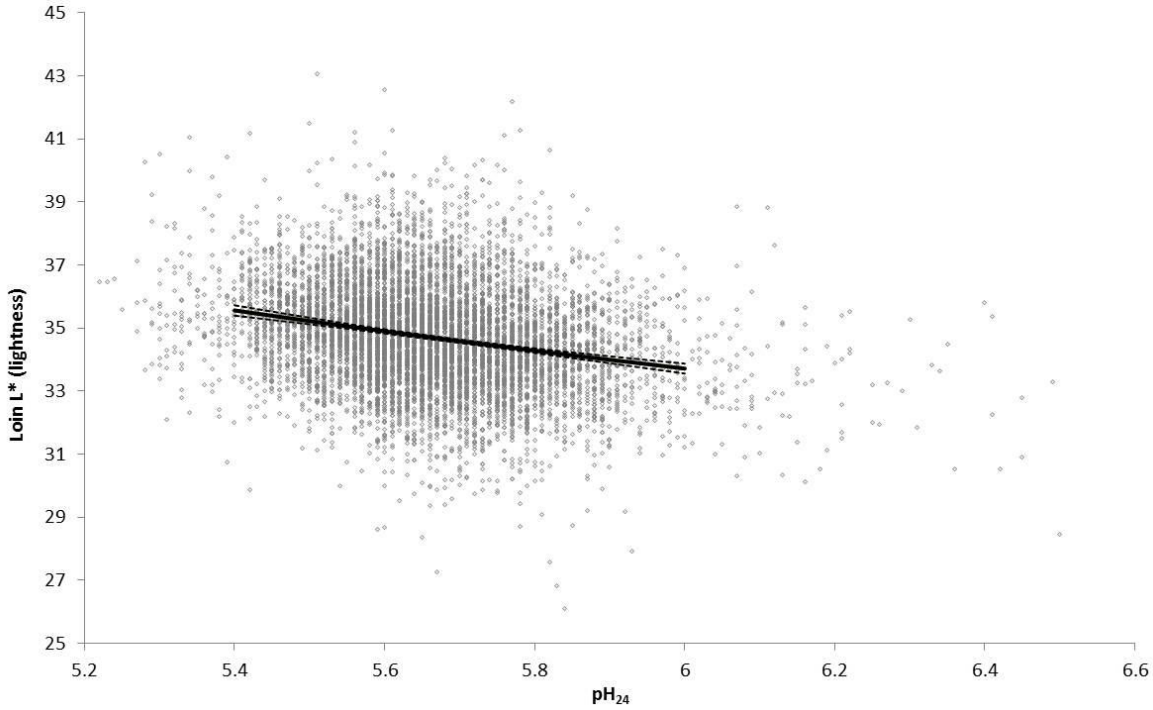

Figure 3-2 Effect of pH_{24} on lamb loin *L** (lightness).

Solid lines represent predicted means and dotted lines represent the standard error of the mean. Icons represent each residual from the predicted means.

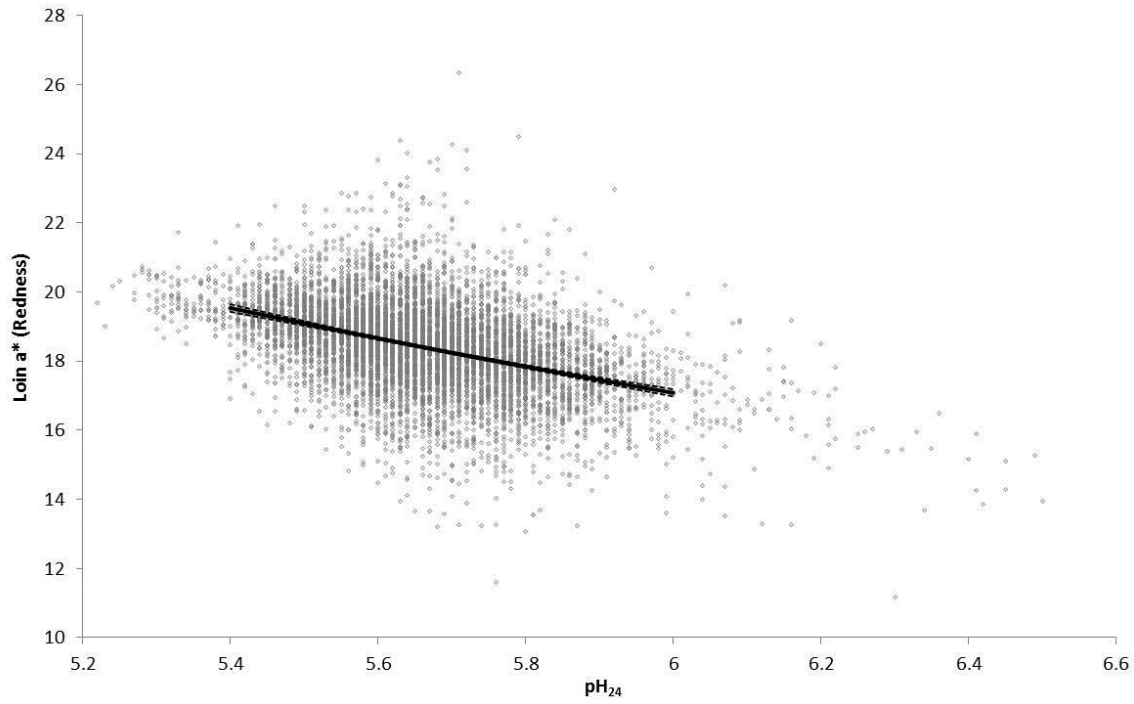

Figure 3-3 Effect of pH_{24} on lamb loin *a** (redness).

Solid lines represent predicted means and dotted lines represent the standard error of the mean. Icons represent each residual from the predicted means.

The myoglobin concentration of the loin muscle was also strongly associated with colour parameters, particularly *L**. Increasing the myoglobin content from 4 to 12 mg/g of muscle reduced loin *L** by 2.8 units ($P < 0.01$; Figure 3-1), the greatest magnitude change in *L** observed in association with changing covariates measured in this study (Figure 3-1). Increasing myoglobin content was associated with a moderate increase in *a** (1.3 units) and chroma (1.2 units) and small decrease in loin *b** (0.2 units) and hue angle (0.9 units) (Figure 3-1).

Muscle iron concentration and ICDH activity had similar associations with *L**, *a**, *b**, hue angle and chroma as myoglobin (Figure 3-1). Increasing iron content from 15 to 30 mg / 100g muscle reduced meat *L** by 2.6 units while increasing ICDH activity from 3 to 9 µmol/min/g of muscle reduced *L** by 1.4 units. Increasing iron and ICDH activity across these same unit changes increased *a** by 0.8 and 0.6 units and chroma by 0.9 and 0.6 units, while decreasing *b** by 0.3 and 0.2 units and hue angle by 1.1 and 1.0 units ($P < 0.01$; Figure 3-1). Increasing muscle zinc concentration from 15 to 35 mg /100g) had a lesser magnitude of effect on fresh colour parameters; reducing *L** by 0.4 units, while increasing *a**, *b**, hue angle and chroma by 0.2, 0.1, 0.5 and 0.1 units ($P < 0.01$; Figure 3-1).

When lamb age at slaughter was added into the model, increasing lamb age at slaughter from 140 to 400 days was associated with a reduction in *L** by 3.1 units and an increase in *a**, *b**, hue angle and chroma by 1.5, 1.1, 2.8 and 1.0 units ($P < 0.01$; Figure 3-1). When the models with pH_{24}, myoglobin, ICDH activity, iron, zinc, or lamb age were corrected for lamb HCWT this did not change the magnitude of their associations with *L**, *a**, *b**, hue angle or chroma.

3.3.3 Summary of effects on meat lightness

The greatest magnitude difference in the lightness of fresh lamb loin was measured between different slaughter groups in this study, followed by differences between sites of lamb production. Differences in loin lightness with increasing lamb age in this study were the next largest magnitude change, followed closely by changes with increasing muscle myoglobin and iron concentration. Differences between years of lamb production accounted for the next greatest difference in lamb loin lightness, followed by changes with increasing pH_{24}, and ICDH activity. Smaller magnitudes of change in loin lightness were seen with increasing lamb HCWT and muscle zinc concentration, the same difference that was observed between sexes of lamb and slightly more than was observed between different sire types, while no significant difference in meat lightness was observed between lambs from different dam breeds. Surprisingly none of the muscle traits measured in this study accounted for the large differences in loin meat lightness observed between different slaughter groups, sites or years of production, when incorporated individually or in combination into the base models, only the effect of lamb age was accounted for by changes in muscle myoglobin concentration.

3.3.4 Summary of effects on meat redness, yellowness, hue angle and chroma

Of all the factors examined in this study, lamb slaughter groups (within any one site and year) were associated with the greatest difference in meat redness and yellowness (Figure 3-1). Alternatively, for meat yellowness and hue angle, site of lamb production had a greater magnitude of effect than slaughter group. Site had the second largest magnitude of effect on meat redness and chroma. Changes in pH_{24} had a greater effect on meat redness, yellowness, hue angle and chroma than any other covariate measured

Table 3-3). pH_{24} was the third greatest factor influencing meat redness, yellowness and chroma, though had marginally less effect on meat hue angle than the year of lamb production.

Differences in meat redness, yellowness and chroma between years of production were similar in magnitude to the effect of changing HCWT, lamb age and muscle myoglobin concentration, with the exception of meat yellowness on which myoglobin had minimal effect on. Similarly myoglobin had little impact on hue angle compared to lamb HCWT. The effect of changing iron concentration and ICDH activity were only 2/3 and ½ that of myoglobin on meat redness and chroma. However, iron and ICDH activity had similar small magnitudes of effect on meat yellowness and hue angle as myoglobin. Changes in zinc concentration had little effect on meat colour, of similar magnitude to the small effect of sire and dam breed, and greater than the smallest recorded differences in meat colour parameters demonstrated between lamb sexes.

3.4 Discussion

3.4.1 Production factor effects on meat lightness

Contrary to our hypothesis, production traits including lamb slaughter group and site of production had a greater effect on the lightness of lamb loin than any muscle trait measured in this study (Table 3-3). The marked differences in meat lightness observed between different production sites, years and slaughter groups was not altered when carcass weight or intrinsic muscle factors including pH_{24} and myoglobin were accounted for in the models, suggesting that other mechanisms are playing an important role in determining the lightness of fresh lamb meat. While the scope and design of this study limit the ability to identify the mechanisms underpinning the important effects of site, year or slaughter group on meat lightness, it is likely a multitude of interacting

factors including animal genetics, production factors and environmental conditions of carcasses are impacting muscle properties and structure- changing light reflection and thus perceived meat lightness without substantial associated changes in meat pH or oxidative capacity (approximated via myoglobin or iron content and ICDH activity).

The grouping of lambs sent for slaughter was associated with the greatest magnitude difference in meat lightness, even when only compared within any one site in any given year of production. This magnitude effect of slaughter group on meat lightness exceeded the effect of site or year of production, of lamb sex or breed type, or of changes in HCWT, pH_{24}, myoglobin or iron concentration, ICDH activity, zinc concentration or lamb age. The slaughter group effect captures variation in slaughter day conditions; from transport and lairage conditions to abattoir processing factors, as well as differences in nutrition leading up to slaughter given these groups of lambs were slaughtered at different times across each year.

No patterns were observed in the mean loin lightness of lamb slaughter groups across each year, between or within different sites of production over the years. However, this study was not designed to examine the impact of nutrition, making meaningful interpretations of possible nutritional influences impractical. Ponnampolam et al. (2014) describes some complexities of the nutritional history of lambs in this study, including differences in the number and variety of pastures and the type of supplementary feed supplied at different sites, at different times of year and in different years of production. Muscle factors determined by nutritional intake such as intramuscular vitamin E concentration, which has been demonstrated to impact meat lightness following blooming (Pearce, Masters et al. 2005), would have varied dramatically between slaughter groups as well as site and years, and thus would account for some of the variation observed between these groups.

Similarly the site of production effect will have captured substantial differences in nutrition, in on-farm production routines and between different abattoirs. The sites in this study were up to 3,500 km apart, meaning substantial climatic variation and thus differences in pasture types, availability, the type and provision of supplementary feed provided to lambs between different sites at different times of year. Factors such as the distance lambs were transported to reach the abattoir also varied substantially between sites. The year of production was associated with less variation in fresh meat lightness than site or slaughter group which is unsurprising given some annual variation in feed types, availability and possibly production factors is expected between different years at any one site, though less than would be expected between different sites and different times of year ie slaughter groups.

Lamb age at slaughter had a substantial impact on fresh loin lightness in this study. Increasing lamb age from 140 to 400 days was associated with a marked reduction in meat lightness. When myoglobin and iron concentrations were accounted for in the models the magnitude of effect of lamb age was reduced by two-thirds, while accounting for ICDH activity and pH_{24} did not change the effect of age on meat lightness. This suggests that the impact of lamb age on fresh meat lightness can be attributed to increasing muscle myoglobin and iron content with age (Kelman, Pannier et al. 2014, Pannier, Pethick et al. 2014) rather than to changes in muscle oxidative capacity (as measured by ICDH activity) or the ultimate pH of the meat.

The impact of lamb breed and sex on fresh meat lightness were small compared to other production factors and intrinsic muscle factors. Terminal sired lambs produced the lightest meat and Merinos the darkest. This sire type effect was accounted for by differences in muscle myoglobin, iron and ICDH activity and not by differences in meat pH_{24}. This is surprising given the perception that Merinos produce darker meat due to

their propensity to produce meat with a higher ultimate pH (Gardner, Pethick et al. 2006). These results instead suggest that differences in oxidative capacity/and or myoglobin concentration underpins the darker meat colour observed in Merino sired lambs. These sire type effects on meat lightness are similar in magnitude to those reported by Teixiera (2005) and far lower than reported by Hopkins and Fogarty (1998), where much smaller numbers of lambs (< 20) represented each breed type. No difference was observed however in the meat lightness of lambs from different dam breeds. Female lambs produced marginally darker meat than males, though the reason is unclear given no intrinsic muscle factor accounted for this effect. Teixiera (2005) also found that female lambs produced darker loin meat, though reported a far larger magnitude of effect, which may be due to the low number of lambs (n = 72) or to the reduced age of the lambs (slaughtered at 90 – 120 days).

3.4.2 Intrinsic muscle factor effects on meat lightness

Contrary to our hypothesis, changing muscle myoglobin content was associated with a greater magnitude of impact on meat lightness than pH_{24} and all other intrinsic muscle factors. The precise reason that increasing myoglobin content reduces lightness and darkens lamb meat may be related to a combination of mechanisms besides the increase in total pigment per se; an associated increase in muscle oxygen consumption, increased density of dark deoxymyoglobin when low oxygen limits the formation of oxymyoglobin, increased absorption of light by myoglobin pigments and thus reduced reflected light and/or a reduced bloom depth resulting in a more superficial dark deoxymyoglobin layer (AMSA 2012). The greater impact of myoglobin compared to pH_{24} on meat lightness in this study supports the phenotypic correlations reported by Mortimer (-0.21 vs -0.12) (2014), though conflicts with the current industry perception that the ultimate pH of meat is the most important factor causing dark red meat. This is

important as the ultimate pH is often used by industry as a rough gauge of the colour that meat will develop once sliced for display. It is possible that the relative importance of ultimate pH could increase in populations where proportionately more of the lambs had an elevated ultimate pH due to poor nutrition and/or stress. However the population represented in this study is a good reflection of Australian production systems, given the diversity of nutritional backgrounds and that most processors follow standard best-practice guidelines for animal welfare. This study demonstrates that myoglobin content is a more important determinant of lamb meat lightness than meat pH_{24} and thus may be a more accurate predictor of dark lamb meat.

The impact of total muscle iron concentration on fresh loin lightness was marked and consistent with the impact of myoglobin. Increasing iron reduced meat lightness to a slightly lesser magnitude than myoglobin, though to a greater extent than pH_{24}. The consistent direction and similar magnitude of impact of iron and myoglobin on loin lightness is unsurprising given the high reported correlations between iron and myoglobin (Mortimer, van der Werf et al. 2014) and their close biochemical association; iron being an essential component of the myoglobin pigment. However, iron is a less sensitive measure of myoglobin pigmentation given that total iron measures capture heme iron within other muscle proteins such as ferritin, as well as non-heme iron, hence the reduced magnitude of impact of iron compared to myoglobin. The impact of iron content on meat lightness in this study is thus likely to reflect changing myoglobin concentration in addition to other unidentified mechanisms.

Myoglobin and iron concentrations are also linked to the proportion of oxidative fibre types and thus the muscle's overall oxidative capacity. Thus the reduction in meat lightness seen with increasing oxidative marker ICDH activity was anticipated. The magnitude of impact of ICDH activity was however only around half that of myoglobin.

This suggests that the strong association between meat lightness and myoglobin concentration relates more to changes in the quantity and density of myoglobin pigment rather than to any associated changes in oxidative metabolism altering oxygen availability within muscle *post-mortem*. This is supported by the fact that the magnitude of impact of myoglobin is reduced by only 5% when variation in ICDH is accounted for in the model. The oxidative capacity of muscle is thus an important factor determining lamb lightness more due to its close association with myoglobin content than due to changes in muscle fibre type and metabolism.

Increasing meat pH_{24} was also associated with a substantial reduction in loin meat lightness in this study where pH_{24} was used as a proxy for ultimate pH. This result is consistent with the majority of literature identifying high ultimate pH as a key cause of dark red meat. This impact was not altered when variation in any other intrinsic muscle factors were accounted for in the pH_{24} models, suggesting that ultimate pH influences fresh meat lightness independent of these factors. Despite its strong impact, pH_{24} failed to account for the effect of any production factors on meat lightness in this study, a surprising finding given that pre-slaughter nutrition and stress are known to impact the ultimate pH reached in meat.

The small increase in meat lightness associated with increasing HCWT of lambs at slaughter could not be accounted for by any intrinsic muscle factors measured in this study, thus the mechanism underpinning this effect is unclear. The fact that slaughter group (and thus lamb age to an extent) was incorporated in the HCWT model and that myoglobin did not account for the impact of HCWT on lightness as it did the impact of age, supports that the influence of HCWT is not a reflection of age differences but some other unknown muscle factor.

Currently processors in the Australian lamb industry have the capacity to know the breed, sex and even the region of production but not the age of the lambs they slaughter. Arguably, the inclusion of slaughter group which would largely account for lamb age in these covariate models may reduce the magnitude of effect that intrinsic muscle factors have on meat lightness at an industry level, particularly myoglobin concentration given its close association with lamb age (Kelman, Pannier et al. 2014). In support of this notion, when slaughter group within year and site of production was removed from the model the impact of changing myoglobin concentration on meat lightness increased by almost 30%.

3.4.3 Production factor effects on meat redness, yellowness, hue angle and chroma

Contrary to our hypothesis, production factors including slaughter group, site and year of production had a greater magnitude of effect on lamb loin redness, yellowness, chroma and hue angle than any intrinsic muscle factor. The precise mechanisms underpinning the impact of these production factors cannot be elucidated in this study though are likely to relate to nutrition, to conditions on the day of slaughter and abattoir factors. The impact of site was particularly large on fresh meat yellowness and thus hue angle and chroma. Given that L^*, a^* and b^* are measured simultaneously using a chromameter, the marked variation seen in yellowness compared to lightness and redness cannot be explained by operator, machine or measurement error. Given the relatively large impact of site relative to year on yellowness it is likely that the type of nutrition fed to lambs prior to slaughter is a particularly important determinant of meat yellowness. Daley et al. (2010) reported that fat yellowness in ruminants is primarily determined by their dietary intake of carotenoids, which varies substantially within and between feed types and thus would have varied considerably between sites in this study.

Increasing lamb age from 140 to 400 days markedly increased meat redness, yellowness, hue angle and chroma in this study, in line with the impact of increasing myoglobin and iron concentration (Figure 3-1). Surprisingly the effects of lamb age on meat redness, yellowness and chroma were less than half the magnitude of effect of age on meat lightness. However this finding is reasonable given that myoglobin and iron had a greater impact on meat lightness relative to redness and chroma than was anticipated in this study. While the increase in meat redness and chroma with increasing lamb age is positive, this is offset by the increase in meat yellowness and hue angle as well as the reduction in meat lightness.

The effect of lamb breed and sex on the meat redness, yellowness, hue angle and chroma were small compared to the impacts of production site, year, slaughter group and intrinsic muscle factors, and compared to previously reported values (Hopkins and Fogarty 1998, Teixeira, Batista et al. 2005). The effects of sire type on meat redness, yellowness, hue angle and chroma were not accounted for by differences in muscle myoglobin, iron or ICDH activity, as was the effect on meat lightness. Nor did any other intrinsic muscle factors examined in this study account for the sire type effect, thus the cause of this effect is unclear. Changes in myoglobin concentration did account for the impact of dam breed on meat redness, though not on yellowness, hue angle or chroma. The marginal increase in chroma of female lamb meat could also be attributed to increased myoglobin concentration.

3.4.4. Intrinsic muscle factor effects on meat redness, yellowness, hue angle and chroma

Contrary to our hypothesis, changes in pH_{24} unexpectedly had a greater magnitude of effect on lamb redness, yellowness, hue angle and chroma than myoglobin or any other intrinsic muscle factor measured (Figure 3-1). In addition to markedly reducing meat

lightness, increasing pH_{24} also negatively impacted on loin meat redness and chroma. Increasing pH_{24} was associated with a substantial reduction in redness (*a**); the measure identified as the single best indicator of consumer acceptability of lamb meat colour by Khliji et al. (2010). Of all the muscle factors examined in this study pH_{24} had the strongest association with redness, its effect on *a** almost double that of the next strongest effects- HCWT, myoglobin and iron concentration. Increasing pH_{24} did reduce meat yellowness markedly, though this measure is thought to have limited importance to consumer acceptability of meat (Khliji, Van de Ven et al. 2010). Of all muscle traits measured, pH_{24} had the greatest magnitude of effect on chroma, which is an important component in the evaluation of meat colour by consumers (AMSA 2012). The impact of pH_{24} on meat redness, yellowness, hue angle and chroma again appears to be independent of other muscle variables including myoglobin, a reasonable finding given the low phenotypic correlation (-0.01) reported between lamb loin pH_{24} and myoglobin concentration (Mortimer, van der Werf et al. 2014).

Increasing muscle myoglobin concentration was associated with a substantial increase in loin redness and chroma. The improved redness and chroma of loin colour with high myoglobin concentration can be clearly attributed to an increased density of the red pigment oxymyoglobin. Again the impact effect of iron concentration on redness and chroma followed the direction of impact to a slightly lesser magnitude than myoglobin concentration, with ICDH activity slightly lesser again. This supports that the effects of iron and ICDH activity on meat redness and chroma also reflect changes to myoglobin pigment concentrations. Increasing myoglobin, iron and ICDH activity had a positive influence by reducing meat yellowness and hue angle, though the magnitude of these effects are small compared to other muscle and carcass traits examined (Figure 3-1). Changes in myoglobin content or any other intrinsic muscle factor did not account for the marked increase in redness and chroma and moderate increase in yellowness and

hue angle associated with increasing lamb HCWT, thus with the reason behind these effects remains unclear.

The improvement in the redness and chroma of high myoglobin meat is offset by the reduced lightness of this meat, which is a particular concern given the importance and prevalence of dark lamb meat. All meat in this study met the consumer acceptability threshold for redness of 9.5, while only 60% of loin samples exceeded the threshold of 34 for meat lightness (Khliji, Van de Ven et al. 2010). This supports anecdotal reports that dark meat is the most important problem causing consumer rejection of freshly cut lamb meat, and suggests that efforts should be focused on increasing meat lightness by reducing muscle myoglobin content in addition to minimising ultimate pH. The high genetic correlation reported between myoglobin concentration and lamb loin lightness (-0.81) suggests that dark meat may be reduced via selection for lower myoglobin concentrations. Reduced muscle myoglobin concentration could be achieved in lambs through a combination of genetic selection for increased muscling and thus reduced proportion of oxidative muscle fibres, as well as by slaughtering lambs at a younger age given the increase in muscle myoglobin content with lamb age (Kelman, Pannier et al. 2014) . This may be further exaggerated by the reduced adrenaline responsiveness and therefore reduced stress susceptibility in high muscling lambs (Martin, McGilchrist et al. 2011) which could lead to a reduction in the incidence of dark cutting.

3.5 Conclusion

Lamb production factors such as slaughter group, site and year of production had far greater magnitudes of impact on all fresh lamb meat colour parameters than any intrinsic muscle factors measured in this study, such as pH or myoglobin concentration, contrary to our expectations. Further investigation is required to better understand how

these production factors are such important determinants of fresh lamb colour independent to the muscle factors most commonly implicated in determining red meat colour. Of the intrinsic muscle factors examined, high pH_{24} of the loin muscle was associated with consistent worsening of fresh lamb meat colour; reducing lightness and substantially worsening redness and chroma. Myoglobin had a greater magnitude impact on meat lightness than pH, though the impact of myoglobin was inconsistent on different fresh colour parameters- high myoglobin markedly decreased thus worsened meat lightness, whilst increasing thus improving meat redness and chroma. Myoglobin and iron, which had consistent impacts though to a slightly lesser magnitude, accounted for the substantial impact of lamb age on fresh lamb colour parameters in this study. Muscle myoglobin content rather than pH also accounted for the darker lamb meat produced by Merino sired lambs. Thus industry focus needs to shift to incorporate consideration of both myoglobin and iron content as well as the ultimate pH of meat in order to better maintain optimal fresh lamb colour development, in addition to further research to better understand how production factors such as slaughter group are having such an important impact on fresh lamb colour independent of the muscle factors measured in this study.

Acknowledgements

The authors gratefully acknowledge the financial support of the Cooperative Research Council for Sheep Industry Innovation (Program 3, Next Generation Meat Quality, project 3.1). Considerable staff and resources were provided at each site of the Information Nucleus Flock by the NSW Department of Primary Industries, University of New England, Department of Primary Industries Victoria, SA Research & Development Institute and the Department of Agriculture and Food WA. The authors also wish to thank the many people who assisted with site supervision, sample

collection and measurement as well as abattoir staff. For the New South Wales sites; Dr. D. Hopkins, Dr. G. Refshauge, Mr. D. Stanley, Ms. S. Langfield, Mrs. T. Bird-Gardiner, Ms. T. Lamb, Mrs. E. Toohey and Mr. M. Kerr. For the Victorian sites; Dr. E. Ponnampalam, Mr. W. Brown, Mr. A Naththarampatha and Mr. M. Kerr. For the Western Australian sites; Dr. Kelly Pearce, Mr. K. Hart, Mr. M. Boyce and Mr. A. Williams.

Chapter 4. Selection for intramuscular fat and lean meat yield will improve the bloomed colour of Australian lamb loin meat

This chapter is published in Meat Science:

Calnan, H.C., Jacob, R.H., Pethick, D.W. and Gardner, G.E. (2016). Selection for intramuscular fat and lean meat yield will improve the bloomed colour of Australian lamb loin meat. Meat Science. 131: 187-195.

Abstract

The colour of bloomed *m. longissimus lumborum* was measured 24 h post slaughter for 8165 lamb carcasses produced over 5 years across 8 sites in Australia. Intramuscular fat across a 2 to 8% range and shortloin fat weight were positively associated with meat lightness (L^*), redness (a^*), yellowness (b^*), hue angle and chroma ($P < 0.01$). Shortloin muscle weight was negativelyassociated with thesemeat colour parameters ($P < 0.01$), although this was largely accounted for by correlated changes in intramuscular fat ($P < 0.01$). The effect of sire breeding values for lamb weight, shortloin muscle depth and fat depth on loin L^*, a^*, b^*, hue angle and chroma were small and varied between lambs of different sire type, dam breed and sex. Thus selection for lean meat yield will have neutral or positive effects on meat colour, while selection for increased intramuscular fat will make the bloomed colour of lamb meat lighter and redder.

4.1 Introduction

The colour of lamb meat on display is critical to retail sales and thus the profitability of the lamb meat industry (Mancini and Hunt 2005). Consumers rely on meat colour as an indicator of quality and freshness. Consumers associate bright red meat with freshness and high quality, and associate unattractive pale or dark meat with imminent spoilage and/or with reduced meat quality (Faustman and Cassens 1990). The discolouration of lamb meat will deter a consumers' purchase, forcing retailers to discount, downgrade or even discard the discoloured product. These practices result in substantial financial losses for the lamb meat industry. However the colour of lamb meat on display is a complex trait that is difficult for producers, processors or retailers to control or predict.

Myoglobin is the pigment primarily responsible for the colour of red meat (AMSA 2012) and is associated with measures of lightness (L^*), redness (a^*), yellowness (b^*), shade of colour (hue angle) and colour saturation (chroma) of lamb meat after blooming (Calnan, Jacob et al. 2016). Blooming is the process whereby the hue angle of meat changes from purple to red and the L^* increases due to myoglobin oxygenation (Brooks 1929). The colour immediately after blooming represents the best colour possible in relation to consumer preference defined by L^* and a^* values for meat on display (AMSA 2012). After blooming the hue angle of meat becomes progressively brown due to oxidation of myoglobin into metmyoglobin. The colour of lamb meat normally remains in this premium "bloomed" state for at least 24 hours under over-wrap (Khliji, Van de Ven et al. 2010) and 8 days under high-oxygen modified atmosphere packaging though the precise time period varies (Channon, Baud et al. 2005).

Factors that increase muscle myoglobin concentration will generally reduce L^*, b^* and hue angle, and increase a^* and chroma values for bloomed meat due to preferential absorption of different wavelengths of light by myoglobin (Calnan, Jacob et al. 2016).

Muscle oxidative capacity as indicated by ICDH activity (Gardner, Hopkins et al. 2007) is strongly related to myoglobin and hence the iron concentration of muscle. While muscle oxidative capacity is associated with mitochondrial oxygen consumption (Klont 1998) which reduces bloom depth and thus *L** and *a**, given the importance of myoglobin in determining the bloomed red meat colour the association between muscle oxidative capacity and myoglobin likely has a greater impact on lamb meat colour. On this basis increasing ICDH activity will be associated with a reduction in lamb meat *L**, *b** and hue angle and increased *a** and chroma. Additionally, any animal factors that influence muscle oxidative capacity are likely to influence the colour of meat after blooming.

Lean meat yield, defined as the weight of saleable meat derived from a carcass, is one such factor that is associated with ICDH activity, iron and myoglobin concentrations (Wegner 2000, Greenwood, Gardner et al. 2006). Sire breeders in Australia have increased the lean meat yield of lamb carcasses, and hence the potential value of carcases, through the use of Australian Sheep Breeding Values (ABSVs) (Anderson, Pethick et al. 2016). The development of breeding values for post-weaning weight (PWT), eye muscle depth (PEMD), and post-weaning c-site fat depth (PFAT) has allowed this selection for lean meat yield. Sire PEMD and PFAT estimates are based on ultrasound measures of *m.longissimus* (loin muscle) depth at the level of the 12^{th} rib and fat depth measured 45 mm from the mid-line over the 12^{th} rib (the c-site) (Fogarty 1995). Lambs from high PWT sires have increased growth rates, while lambs from high PEMD sires have increased muscle size particularly of the loin muscle (Gardner, Williams et al. 2010) and lambs from low PFAT sires have reduced carcass fatness (Gardner, Williams et al. 2010).

Selection for lean muscle growth has indirectly changed muscle fibre morphology and reduced muscle ICDH activity (Greenwood, Gardner et al. 2006), myoglobin and iron concentrations in slaughter lambs. The use of high PWT sires has reduced muscle oxidative capacity due to a reduction in relative maturity of lambs at the time of slaughter (Hall 2000). Selection for high PWT increases the mature size of the lambs, which are then relatively less mature when slaughtered at a set carcass weight (Huisman and Brown 2008). Given that maturity is associated with increased carcass fatness and more oxidative muscle fibres; these less mature lambs have relatively leaner carcasses with reduced muscle oxidative capacity (Brandstetter, Picard et al. 1998). Selection for sires with high PEMD and low PFAT breeding values has reduced lamb muscle oxidative capacity; with higher proportions of glycolytic type IIX muscle fibres (Greenwood, Gardner et al. 2006), reduced oxidative muscle fibres (Gardner, Pethick et al. 2006) and reduced myoglobin and iron concentrations (Pannier, Ponnampalam et al. 2010, Kelman, Pannier et al. 2014). Selection for lean meat yield may therefore be expected to increase L^*, b^* and hue angle values and reduce a^* and chroma values of bloomed lamb meat.

Sire selection for increased lean meat yield has also had the unintended impact of reducing IMF content and thus the eating quality of lamb meat (Pannier, Pethick et al. 2014). Changes in IMF concentration may also impact the bloomed colour of lamb meat. Mortimer, van der Werf et al. (2014) and Wolcott, Johnston et al. (2009) reported in positive correlations between IMF and meat L^* in lamb and beef, likely due to increased reflection of light from white fat contributing to L^* which is measured on a scale of black (0) to white (100) (CIE 1976). In contrast, over differing ranges in IMF Lorentzen and Vangen (2012) and Fiems (2000) reported that with increasing IMF in lamb and beef that meat L^* reduced and a^* increased; potentially due to the positive association between IMF and muscle oxidative capacity (Hocquette, Jurie et al. 2003).

With greater myoglobin concentration more light is absorbed, the greater the oxygen consumption of the meat and the darker the colour of the meat (low *L**). So while selection for lean meat yield is expected to reduce IMF concentration, the prevailing influence of the associated change in IMF concentration on the bloomed colour of lamb meat is unclear.

Given the importance of colour in driving lamb meat sales and profitability, it is vital to understand the influence that selection for lean meat yield and subsequent changes in IMF concentration is having on lamb meat colour. This study provides a comprehensive analysis of associations between IMF, genetic and phenotypic indicators of lean meat yield and fresh lamb loin colour parameters, reporting the magnitudes of impact that selection for increased IMF and lean meat yield will have on these parameters. We hypothesised that the progeny of high PWT, high PEMD and low PFAT sires will produce lamb loins with increased *L**, *b** and hue angle values, and reduced *a** and chroma values due to reduced muscle oxidative capacity. Additionally, that reducing IMF concentration in lamb loins will increase surface *L**, *b** and hue angle, and reduce *a** and chroma.

4.2 Materials and Methods

4.2.1 Experimental design, slaughter and carcass measurement details

Lambs (n = 8165) were produced as part of the Sheep CRC INF experiment, over a 5 year period (2007 - 2011) at 8 sites across Australia. This experiment has been comprehensively described previously (AEC numbers 1-7-02, 1-10-1 and 3-10-13) (Fogarty, Banks et al. 2007, van de Werf, Kinghorn et al. 2010). Fresh meat colour was measured from lambs produced at all eight sites; Cowra, Kirby and Trangie in New

South Wales, Hamilton and Rutherglen in Victoria, Struan and Turretfield in South Australia, and Katanning in Western Australia.

The lambs were the progeny of 426 different sires, all with established ASBVs for post-weaning loin muscle depth (PEMD), post-weaning c-site fat depth (PFAT) and post-weaning weight (PWT). Sire breeding values are derived from measurements on the individual animals, the performance of their relatives, and appropriate genetic parameters (Fogarty 1995). Sire PFAT values are calculated from ultrasound measurements of fat depth at the c-site (45 mm from the mid-line over the 12^{th} rib) and adjusted to 60 kg live weight (Fogarty 1995). Sire PEMD values are based on ultrasound measurement of the depth of the loin muscle at the level of the 12^{th} rib, adjusted for live weight (Hall, Gilmour et al. 2002), while PWT estimates are based on lamb live weights corrected to 225 days of age. ASBVS are expressed as either positive or negative deviations from an average. Sire ASBVs are calculated for each breed type, thus different ranges in ASBVs exist between sire breed types.

The sires comprised Terminal sire types (Hampshire Down, Ile De France, Poll Dorset, Southdown, Suffolk, Texel and White Suffolk), Maternal sire types (Bond, Booroola, Border Leicester, Coopworth, Corriedale, Dohne Merino, East Friesian, Prime SAMM and White Dorper) and Merino sire types (Merino and Poll Merino). Around 90 sires were used each year to inseminate dams across all sites in the study. Semen from all three sire types was used to artificially inseminate Merino dams, while only Terminal sires were used to inseminate crossbred ewes (eg Border Leicester x Merino dams). Maternal and Merino sired lambs sent to slaughter comprised very few females (which were retained for breeding purposes), meaning effective comparisons between sexes could only be made within the Terminal sired lamb groups. Hence the lambs comprised six sire type, dam breed and sex combinations; Maternal sired males from Merino dams

(n=1555), Merino sired males from Merino dams (n=1292), Terminal sired males from Merino dams (n=1195), Terminal sired females from Merino dams (n=1153), Terminal sired males from Crossbred Merino dams (n=1518) and Terminal sired females from Crossbred Merino dams (n=1452).

The lambs were grazed on pasture that was supplemented with grain, hay or feedlot pellets when low pasture supply limited lamb growth. Management practices and carcass processing were standardised as far as possible to examine the effects of factors such as production site, year and slaughter group on lamb meat colour. These effects have been described in a previous publication (Calnan et al., 2016), while several publications provide details of breed types used, lamb feeding and management practices in this experiment (van de Werf, Kinghorn et al. 2010, Ponnampalam, Butler et al. 2012, Ponnampalam, Butler et al. 2014, Calnan, Jacob et al. 2016). At each site lambs were consigned to smaller groups to be killed on the same day (slaughter groups). There were a total of 125 slaughter groups in this study containing 65 lambs on average. Lambs were allocated to slaughter groups based on live weights, targeting a carcass weight of 21- 22 kg, so ranged in age from 134 to 504 days at the time of slaughter. Lambing dates were clustered because mating was done using artificial insemination thus the range of lamb ages within slaughter groups was 11 days and less than expected with natural mating. The day prior to slaughter the lambs were held for 6 hours in a small paddock without food or water then weighed and transported to a commercial abattoir. Trucking distances varied substantially between different production sites but were consistent within production sites. At the abattoir, lambs were held in lairage overnight with access to water but no food before slaughter the following day. All carcasses were subjected to medium voltage electrical stimulation (Pearce, van de Ven et al. 2010) and trimmed according to AUS-MEAT specifications (Anonymous 2005).

Carcasses were weighed after slaughter to determine the HCWT. Carcasses were then chilled overnight to a temperature of 3 - 4 °C prior to sampling. The *m. longissimus thoracis et lumborum* (loin muscle) was removed (caudal from the 12th rib region to the lumbar sacral junction) and weighed separately to the overlying subcutaneous fat layer (shortloin muscle weight and shortloin fat weight). The IMF content of the loin was determined using a 40 g sample of muscle excised from the caudal (lumbosacral) aspect, from which all visible subcutaneous fat and silver skin were removed prior to dicing. The muscle was then stored in 50ml collection tubes at -20°C prior to freeze drying using a Cuddon FD 1015 freeze dryer (Cuddon Freeze Dry, NZ). The percentage of IMF was then determined using a near infrared procedure in a Technicon Infralyser 450 (19 wavelengths) using the method described by Perry et al. (2001) and was expressed as a percentage of wet tissue weight.

Myoglobin concentration, iron concentration, ICDH and pH at 24 hours (pH_{24}) were also measured in each lamb loin muscle. Details on the methods of collection and measurement of these parameters has been previously described (Calnan, Jacob et al. 2016).

4.2.2 Meat colour measurement

The colour of each *m. longissimus* (loin) was measured at 24 hours post slaughter after the cut surface had been allowed time to bloom (oxygenate). The loin muscle was sliced perpendicular to muscle at the level of the 12th rib to allow the removal of the shortloin muscle. The cut loin meat surface (remaining on the carcass) at the level of the 12th rib was then allowed 30 – 40 minutes exposure to air at the chiller temperature (2 – 4 ° C), before surface colour was measured using a Minolta Chromameter, Model CR – 400 (Konica Minolta Optics, Inc.). A different machine was used at each abattoir.

Each Minolta chromameter was fitted with a closed cone, set on a "D65" illuminant, using a 2° standard observer and 8 mm measurement area (aperture). The aperture was held directly against the meat surface for colour measurements. The chromameter was fitted with a CR-A33a or CR-A33f light projection tube with a glass 'shield' fitted to the base of the measuring head. Each chromameter was calibrated prior to use within the chiller where measurements were subsequently taken using a white tile according to manufacturer's instructions.

The chromameter measured the colour of the freshly sliced meat surface according to the Commission International de l'Eclairage (CIE) *L*a*b** system (CIE 1976). According to this system *L** or lightness represent dark to light on a scale of 0 to 100; *a** or redness represents green to red on a scale of -60 to +60; and *b** or yellowness represents blue to yellow on a scale of -60 to +60. Hue angle was calculated manually (arctangent (*b**/*a**)) with values from 0-360° representing a colour shade based on a colour circle, where 0° (= 360°) represents red, 90° yellow, 180° green and 270° blue hues. Thus in the context of meat colour, hue angle values closest to 0° or 360° are considered preferable to consumers. To correct for negative *b** values creating negative hue angles, 360 was added to all hue angle values to allow analysis, before being subtracted from any values exceeding 360, according to the method of McGuire (1992). Chroma or the colour saturation index of the meat surface was calculated using *a** and *b** measures with the equation: $\sqrt{(a^{*2} + b^{*2})}$ (AMSA, 2012).

Three replicate measurements were obtained of each meat surface with the chromameter head rotated by 45 ° for each measure and with an effort made by the operator to avoid areas of dense connective tissue. The three measures from each loin sample were then averaged for analysis.

4.2.3 Statistical analysis

Base models for *L**, *a**, *b**, hue and chroma have been detailed in previously published work (Calnan, Jacob et al. 2016). These models contained fixed effects for site, year, slaughter group within site and year, sex and dam breed within sire type and site by year, as well as random effects for sire and dam by year. The analysis in this manuscript made use of these previously developed base models, to test the effects of phenotypic carcass measurements and ASBVs on the colour parameters.

For *L**,*a** and *b** this analysis was carried out in two parts. Firstly a multivariate analysis using *L**, *a** and *b** as the dependant variables was used in SAS (Version 9.1, SAS Institute, Cary, NC, USA) to establish a unique model for each carcass measurement. These carcass measurements included HCWT, IMF concentration, shortloin muscle weight, and shortloin fat weight. Each measurement was incorporated separately as a covariate into the base model for *L**, *a** and *b**, along with the squared term of the covariate and all relevant first order interactions between the covariate and fixed effects. Non-significant ($P > 0.05$) terms were removed in a stepwise manner. Secondly, these unique models for each covariate (with and without HCWT) were then tested in separate linear mixed effects model for each of *L**, *a** and *b** to examine the association between each covariate and each colour parameter. IMF was incorporated separately into each colour model, while shortloin muscle weight and shortloin fat weight were each analysed in conjunction with HCWT to ensure the impact of composition (relative muscling or fatness) on *L**, *a**, and *b** was represented. IMF was also tested in each model in conjunction with HCWT, to ensure that any observed associations were not simply reflecting changes in HCWT.

The same two part approach was taken to test the associations between ASBVs PEMD, PFAT and PWT with *L**, *a** and *b**. All three ASBVs, their squared terms and relevant

interactions with fixed effects were regressed in a multivariate regression analysis of *L**, *a** and *b**. Non-significant terms ($P > 0.05$) were removed to form a base model that was then applied to linear mixed effects models to test for ASBV associations with dependent variables separately (*L**, *a** and *b**), with the inclusion of sire and dam by year as random terms. Given the correlations that exist between the breeding values in this data set (PWT vs PEMD = 0.25; PWT vs PFAT = 0.32; PEMD vs PFAT = 0.20) this process was repeated with each breeding value included one at a time to test the independence of their effects. The effects of the ASBVs were then tested in models corrected for HCWT, shortloin weight, shortloin fat weight and IMF.

For hue angle and chroma, linear mixed effects models were used directly to test their associations with phenotypic carcass covariates and ASBVs. Non-significant fixed and covariate terms, interactions between them and squared covariate terms ($P > 0.05$) were removed in a stepwise fashion using a linear mixed effects model. Again IMF was incorporated separately, while shortloin muscle weight and shortloin fat weight each analysed in models with HCWT incorporated. These final models were tested in linear mixed effects models to output associations between various covariate effects and hue angle and chroma. Other muscle measurements including myoglobin concentration, iron concentration and pH_{24} were also incorporated as covariates into all these models to test biological associations underpinning the effects of IMF, shortloin muscle weight, shortloin fat weight and ASBVs on lamb loin *L**, *a**, *b**, hue angle and chroma.

4.3 Results

The number, mean, standard deviation and range for the fresh colour data, carcass covariates and the sire breeding values within each sire type are shown in Table 4-1. The base models described 65%, 71%, 94%, 96% and 66% of the variance in *L**, *a**,

*b**, hue angle and chroma respectively. The *b** values were positively associated with *L** and *a** with partial correlation coefficients of 0.4 and 0.6 respectively, though there was no correlation between *L** and *a** (0). The impact of the fixed production effects (site and year of production, slaughter groups within site and year, sire types, dam breed and lamb sex) on lamb loin *L**, *a**, *b**, hue angle and chroma have been published previously, and are reported in Figure 4-1 for comparison (Calnan, Jacob et al. 2016). Their impact is reported as the magnitude difference between the minimum and maximum predicted means, for example there was a maximum difference of 4.14 in the predicted mean *L** between different sites of lamb production (Figure 4-1). The impact of covariate effects such as muscle myoglobin concentration and pH_{24} on lamb loin *L**, *a**, *b**, hue angle and chroma have been previously published (Calnan, Jacob et al. 2016).

Table 4-1 Description of lamb loin fresh colour variables, covariate data and the Australian sheep breeding values for Maternal, Merino and Terminal sires.

	Number lambs	Mean	St. Dev.	Range
*L**	8165	34.9	3.07	24.4 – 46[c]
*a**	8165	18.4	2.35	9.8 – 30[c]
*b**	8165	3.77	4.13	-5.4 – 19.3[c]
Hue angle	8165	11.3	12.5	-18.6 – 40.3[c,d]
Chroma	8165	19.3	2.36	10.1 – 32.2[c]
Covariates (units):				
IMF (%)	5035	4.20	1.03	1.59 – 10.45
Shortloin muscle weight (g)	8045	360	85.8	140 – 1110
Shortloin fat weight (g)	8038	198	104	10 – 880
Hot carcass weight^ (kg)	8074	23.1	3.72	12.5 – 40.0
Myoglobin^ (mg/g muscle)	5032	6.6	1.88	2.2 – 15.6
Iron^ (mg/100g muscle)	5016	20.4	3.66	8.1 – 45.1
Isocitrate dehydrogenase activity^ (µmol/min/g)	2881	5.0	1.61	1.0 – 11.3
pH_{24}^	8149	5.7	0.14	5.2 – 6.9
Maternal sire estimates (82 sires)	1225			
PEMD (post-weaning eye muscle depth)		0.17	0.66	-1.44 – 1.82
PFAT (post-weaning fat depth)		-0.02	0.85	-1.61 – 2.56
PWT (post-weaning weight)		5.26	2.62	-3.66 – 10.5
Merino sire estimates (161 sires)	1210			
PEMD		0.06	1.01	-2.03 – 2.69
PFAT		-0.15	0.70	-1.89 – 2.03
PWT		2.08	3.11	-5.0 – 10.8
Terminal sire estimates (183 sires)	5296			
PEMD		1.12	1.19	-2.9 – 4.92
PFAT		-0.76	0.79	-2.4 – 2.27
PWT		12.5	2.49	1.13 – 18.29

^ Description of covariate data published previously (Calnan et al., 2016).

[c] Scales of *L**, *a**, *b**, hue angle and chroma values: *L**: -100 to 100, *a** and *b**: - 60 to 60, hue angle: 0 to 360°, chroma: 0 to 60.

[d] Hue angles between 340 and 360° have been displayed as negative values (= minus 360) to show a continuous unit range (ie 341.4° depicted as – 18.6).

4.3.1 Effect of IMF, shortloin muscle and shortloin fat weight on *L**, *a**, *b**, hue angle and chroma

Lamb loin IMF concentration was positively associated with *L**, *a**, *b**, hue angle and chroma ($P < 0.01$, Figure 4-1). When IMF content increased from 2 to 8 % *L** increased by 3.1 units; *a** increased by 1.3 units; *b** increased by 1.7 units; hue angle increased by 4.0 units and chroma increased by 1.4 units (Figure 4-1, Table 4-2). When HCWT, myoglobin concentration, iron concentration and pH_{24} were included separately into each model examining the influence of IMF on lamb loin colour ($P < 0.01$), there was little change in the magnitude of effect of IMF on *L**, *a**, *b**, hue angle and chroma (Table 4-2).

With HCWT accounted for in the model, shortloin muscle weight showed a significant but quantitatively smaller negative association with loin *L**, *a**, *b**, hue angle and chroma ($P < 0.01$, Figure 4-1). Increasing shortloin muscle weight from 100 to 500 g produced a 0.35, 0.65, 0.35, 0.75 and 0.5 unit decrease in *L**, *a**, *b**, hue angle and chroma (Figure 4-1, Table 4-2). Though increasing shortloin fat weight (with HCWT accounted for) caused a larger increase in *L**, *a**, *b**, hue angle and chroma ($P < 0.01$, Table 4-2). Increasing shortloin fat weight across a range of 100 to 500 g caused a 0.9, 0.8, 1.2, 2.4 and 1.0 unit increase in *L**, *a**, *b**, hue angle and chroma (Figure 4-1, Table 4-2). Incorporating myoglobin concentration into each model, along with HCWT, did not substantially alter the impact of shortloin muscle weight on meat colour parameters, though did reduce the impact of shortloin fat weight on loin *L** and *a** ($P < 0.01$, Table 4-2). However, incorporating IMF % in addition to HCWT did substantially change the impact of shortloin muscle weight and shortloin fat weight on lamb loin *L**, *a**, *b**, hue angle and chroma ($P < 0.01$, Table 4-2).

	*L**	*a**	*b**	*Hue*	*Chroma*
a) Fixed Effects : Difference between minimum and maximum predicted mean (Calnan et al., 2016)					
Site of production	4.14	3.70	8.33	26.9	2.58
Year of birth	2.43	1.30	1.57	5.0	1.55
Slaughter group	6.51	5.72	7.91	22.5	5.76
Sire type	0.33	0.18	0.16	0.40	0.19
Dam breed	$0.03^{n.s}$	0.18	0.22	0.60	0.21
Sex	0.43	0.10	$0.04^{n.s}$	$0.03^{n.s}$	0.13
b) Covariate Effects : Associations & difference in the predicted mean					

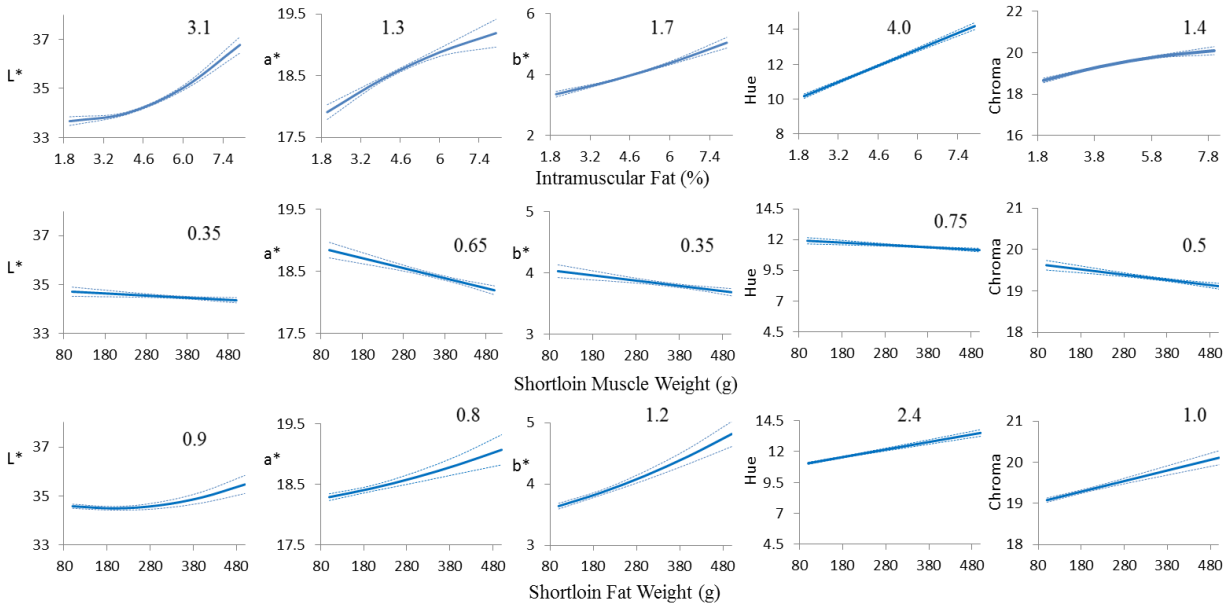

Figure 4-1 The impact of IMF, shortloin muscle and fat weight on lamb loin *L**, *a**, *b**, hue angle and chroma.
a) Difference between the minimum and maximum predicted mean lamb loin *L**, *a**, *b**, hue angle and chroma between different sites and years of production, slaughter groups within site and year, sire types, dam breeds and sexes within sire type covariate effects include **b)** The predicted association between *L**, *a**, *b**, hue angle and chroma and covariates effects: IMF percentage, shortloin muscle weight and shortloin fat weight. Solid lines within figures represent predicted means while dotted lines represent the standard error of the mean. The number above each figure represents the unit change in *L** (lightness), *a** (redness), *b** (yellowness), hue angle and chroma across the range in each covariate. All values represent a significant ($P < 0.05$) association between a fixed or covariate effect and *L**, *a**, *b**, hue angle or chroma, except where $^{n.s.}$ denotes a non-significant association ($P > 0.05$).

Table 4-2 The magnitude impact on *L**, *a**, *b**, hue angle and chroma values across the range in IMF , shortloin muscle weight and shortloin fat weight, alone and with incorporation of additional covariates.

Additional covariates include IMF with myoglobin, iron or pH_{24}, and shortloin muscle and fat weight with hot carcass weight in addition to IMF or myoglobin. All values represent a statistically significant effect ($P < 0.05$) of IMF, shortloin muscle weight or shortloin fat weight on *L** (lightness), *a** (redness), *b** (yellowness), hue angle and chroma.

	*L**	*a**	*b**	Hue angle	Chroma
IMF (2-8%)	**3.1**	**1.3**	**1.7**	**4.0**	**1.4**
+ hot carcass weight	3.0	1.0	1.6	3.8	1.2
+ myoglobin concentration	3.0	1.2	1.65	4.1	1.4
+ iron concentration	3.0	1.25	1.7	4.3	1.55
+ pH_{24}	3.2	1.15	1.7	4.0	1.3
Shortloin muscle weight (100-500g)	**-0.35**	**-0.65**	**-0.35**	**-0.75**	**-0.5**
+ myoglobin concentration	-0.55	-0.6	-0.4	-0.7	-0.6
+ IMF %	0.4	-0.3	0.1	0.4	-0.35
Shortloin fat weight (g) (100-500g)	**0.9**	**0.8**	**1.2**	**2.4**	**1.0**
+ myoglobin concentration	0.45	0.5	1.2	2.7	0.8
+ IMF %	0.1	0.5	0.9	1.8	0.6

4.3.2 Effect of sire breeding values on *L**, *a**, *b**, hue angle and chroma

Sire breeding values for PEMD and PFAT were associated with lamb loin *L**, *a**, *b** and chroma, while only PEMD was associated with hue angle ($P < 0.05$, Table 4-3). There was no association between PWT and any of the meat colour parameters measured ($P > 0.05$). The associations between breeding values and meat colour parameters differed between the six different sire type, sex and dam breed combinations ($P < 0.05$, Table 4-3), with the exception of the consistent effect of PFAT on meat *L** and the effect of PEMD on meat hue angle ($P < 0.05$, Table 4-3).

Increasing PEMD sire estimates was associated with increased meat *L** in two of the six breed type and sex combinations ($P < 0.05$, Table 4-3). When myoglobin concentration was incorporated into the model the effect of PEMD on meat *L** became consistent across sire types; increasing loin *L** by 1.1 units across an increasing PEMD range of -2.9 to 4.9 ($P < 0.05$). The effect of PEMD estimates of loin *a** and *b** were marginal. PEMD was associated with *a** in two of the six breed type and sex combinations, though these associations were opposing, while PEMD was positively associated with loin *b** in only one breed type and sex combination ($P < 0.05$, Table 4-3). Incorporating myoglobin or iron concentration did not alter the impact of PEMD on loin *a** in Terminal lambs, though did account for the impact of PEMD on *a** in Merino lambs ($P > 0.05$). The impact of PEMD on loin *b** became independent of breed type when myoglobin, iron or IMF were incorporated; increasing PEMD from -2.9 to 4 increasing loin *b** by 0.75, 0.55 and 0.55 units ($P < 0.05$). Incorporating pH_{24} did not however change the impact of PEMD on *b** (Table 4-3).

Sire PEMD estimates had a more consistent association with chroma and particularly hue angle. Sire PEMD values were positively associated with chroma in three of the six

breed type and sex combinations, though a negative association was observed in one breed type and sex combination ($P < 0.05$, Table 4-3). Myoglobin, iron and IMF each accounted for these effects of PEMD on meat chroma ($P > 0.05$). Increasing PEMD increased loin hue angle independent of lamb breed type and sex ($P < 0.05$, Table 4-3), and the magnitude of this impact increased to 1.3, 1.1 and 1.0 units when IMF, myoglobin and iron concentration were accounted for.

Higher PFAT estimates were associated with reduced loin *L** independent of breed type and sex combination ($P < 0.05$, Table 4-3), meaning that sire selection for reduced PFAT will be associated with lighter lamb loin. The association between PFAT sire estimates and lamb loin *a**, *b** and chroma were less consistent, while PFAT had no association with loin hue angle ($P > 0.05$, Table 4-3). Reducing PFAT estimates reduced lamb loin *a** in the majority of lamb breed type and sex combinations. PFAT and *a** were positively associated in four of the six combinations, though a negative association was observed in one breed type and sex combination ($P < 0.05$, Table 4-3). Incorporation of myoglobin and iron concentration each accounted for the effect of PFAT on meat *L** and *a** ($P > 0.05$). IMF did not alter the impact of PFAT on loin *L** ($P < 0.05$), though did account for the effect of PFAT on loin *a**. A positive association between PFAT and *b** was found in two of the breed type and sex combinations, though PFAT was negatively associated with *b** in one breed type and sex combination ($P < 0.05$, Table 4-3). The positive association between PFAT and chroma was consistent though only observed in three of the breed type and sex combinations ($P < 0.05$, Table 4-3). The effect of PFAT on meat *b** and chroma was accounted for when myoglobin, iron or IMF concentration were incorporated ($P > 0.05$).

Table 4-3 Difference between the minimum and maximum predicted lamb loin *L**, *a**, *b**, hue angle and chroma measured across the range of sire ASBVs for PEMD and PFAT for each sire type, sex and dam breed combination.

The range of sire ASBVs (Australian sheep breeding values) differed between sire types. Sire ASBV effects on meat colour were dependent on lamb sire type, sex and dam breed, except the impact of PEMD (post-weaning eye muscle depth) on hue angle and PFAT (post-weaning c-site fat depth).on *L**. *L** represents lightness, *a**: redness, *b**: yellowness.

ASBV (range)	Sire type (ASBV range)	Sex Dam breed	*L**	*a**	*b**	Hue angle
PEMD (-2.9 - 4.9)						0.72^
	Maternal (-1.4 – 1.8)	M Merino	1.25	n.s.	0.46	0.30^
	Merino (-2.0 – 2.7)	M Merino	n.s.	0.62	n.s.	0.43^
	Terminal (-2.9 – 4.9)	F Cross bred	n.s.	-0.86	n.s.	0.72^
	Terminal (-2.9 – 4.9)	F Merino	n.s.	n.s.	n.s.	0.72^
	Terminal (-2.9 – 4.9)	M Cross bred	0.95	n.s.	n.s.	0.72^
	Terminal (-2.9 – 4.9)	M Merino	n.s.	n.s.	n.s.	0.72^
PFAT (-2.4 - 2.6)			-0.64^			n.s.
	Maternal (-1.6 – 2.6)	M Merino	-0.54^	n.s.	-0.50	n.s.
	Merino (-1.9 – 2.0)	M Merino	-0.50^	-0.71	n.s.	n.s.
	Terminal (-2.4 – 2.3)	F Cross bred	-0.60^	0.96	0.70	n.s.
	Terminal (-2.4 – 2.3)	F Merino	-0.60^	0.71	n.s.	n.s.
	Terminal (-2.4 – 2.3)	M Cross bred	-0.60^	0.60	0.43	n.s.
	Terminal (-2.4 – 2.3)	M Merino	-0.60^	0.56	n.s.	n.s.

^ represents an ASBV effect independent of sire type, sex and dam breed. For these effects, apparent differences between sire types are due to differences in the sire type range for each ASBV.

n.s. represents a non-significant association ($P > 0.05$).

All values represent a statistically significant ABSV effect on a colour parameter ($P < 0.05$).

4.4 Discussion

4.4.1 Effect of IMF concentration on lamb loin *L**, *a**, *b**, hue angle and chroma

IMF concentration increased loin meat *L** in this study, contrary to our hypothesis. The magnitude of effect on meat *L** was greater (3.1 units) across the range of IMF concentration than previously reported across the range in myoglobin, iron or pH_{24} (2.8, 2.6 and 1.9 units) (Calnan, Jacob et al. 2016). The positive association between IMF and meat *L** in this study has important industry significance given the importance of these traits to the consumer preference for lamb meat in terms of visual appeal and taste. The unexpected increase in *L** with high IMF suggests that reflection of light from white fat is having a greater influence on meat *L** than any change in light absorption associated with high myoglobin or iron concentrations. The magnitude of the effect of IMF on loin *L** was reduced by less than 10% when loin myoglobin or iron concentrations were accounted for in the models of this study, supporting that IMF is influencing meat lightness independent to any associated changes in muscle oxidative capacity.

In line with our hypothesis, increasing IMF concentration in the lamb loin was associated with increased *a**. However, the magnitude of effect of IMF on *a** was reduced by only 10% when muscle myoglobin concentration was accounted for in the model, suggesting that changes in myoglobin concentration account for only a small portion of this effect. The ultimate pH of lamb meat (or pH measured at 24 hours post slaughter – pH_{24}) is an important muscle factor impacting lamb loin *a** on display (Calnan, Jacob et al. 2016). Yet accounting for pH_{24} in these models did not alter the effect of IMF on lamb loin *a**, leaving the principal mechanism underpinning this

advantageous association unclear. Swatland (2003) suggested that structural differences between fibre types including mitochondrial density may account for differences in muscle oxidative capacity independently of myoglobin concentration. While changes in IMF concentration had the same magnitude impact on meat *a** as myoglobin (1.3 units) and a lesser magnitude impact than changing pH_{24} (2.5 units) (Calnan, Jacob et al. 2016), muscle traits such as pH_{24} and myoglobin are complex and difficult to reliably control. IMF concentration alternatively has the potential for independent genetic selection, with a sire ASBV for IMF having been recently developed for the Australian lamb industry. Great potential lies in this ability to independently select for increased IMF and thereby to improve the bloomed colour and taste qualities of lamb meat for Australian consumers (Khliji, Van de Ven et al. 2010). However these gains associated with increased IMF concentration may be offset by worsened meat colour stability (Faustman, Sun et al. 2010, Calnan, Jacob et al. 2014).

Increased IMF concentration was also associated with increased *b** of loin meat in this study. This positive association supports the findings of Mortimer et al. (2014) who reported a high positive genetic correlation of 0.81 between IMF content and *b** in lamb meat. The mechanism behind this association may simply relate to the white/yellow colour of IMF absorbing blue light wavelengths and thus increasing *b** given that *b** values are measured along a scale from blue (-60) to yellow (+60), rather than a change in the *b** of the muscle tissue itself. The increase in *b** with increasing IMF could also be related to the strong positive associations of *b** values with *L** and *a** values. The importance of *b** to consumer appeal and approval of lamb meat colour is unclear, though *b** measures are used in combination with *a** measures to determine meat hue angle and chroma.

In line with the positive association between IMF concentration and both *a** and *b**, increased IMF also increased hue angle and chroma values. The increase in hue angle value with high IMF results in the shade of meat colour being slightly more yellow (90°) than red (0°), despite the increased *a** associated with high IMF. This suggests that increasing IMF is having a relatively greater influence on meat yellowness than redness, though the importance of this change is difficult to gauge given that the importance of lamb meat yellowness measured by *b** or hue angle values has not been well established. Alternatively, the increase in chroma seen with increasing IMF concentration will improve meat colour on display- as chroma represents the colour saturation or brightness of bloomed meat colour which is important to consumer appeal (Khliji, Van de Ven et al. 2010).

Overall increasing the level of IMF within lamb loins has a positive effect on bloomed meat colour; increasing *L**, *a** and chroma - three colour parameters that have been linked to consumer approval of displayed lamb meat colour (Khliji, Van de Ven et al. 2010). These results are encouraging and they suggest that current industry selection for higher IMF levels will not only improve meat taste qualities but will reduce the incidence of dark or discoloured lamb meat that has reduced retail value.

4.4.2 Effect of sire PEMD estimates on lamb loin *L**, *a**, *b**, hue angle and chroma

Selection for high sire PEMD values had inconsistent effects on bloomed meat colour parameters other than hue angle in this study. Increasing sire PEMD estimates was associated with increased lamb loin *L** in Maternal sired lambs and Terminal sired male lambs from crossbred Merino dams only. This positive association aligns with our hypothesis that increasing lean meat yield in lambs via selection for high sire PEMD will increase *L** due to reduced muscle oxidative capacity and thus myoglobin

concentrations. PEMD however was not associated with meat *L** in Merino sired lambs or Terminal sired lambs that were female or from Merino dams. Kelman et al. (2014) found that increasing Maternal and Terminal PEMD estimates were associated with reduced myoglobin concentrations in their progeny, consistent with other reports (Gardner, Pethick et al. 2006). However contrary to expectations high Merino sire PEMD estimates were associated with increased myoglobin concentration in lamb loin muscle (Kelman, Pannier et al. 2014). This increase in myoglobin concentration with increasing PEMD in Merino breed types may explain the lack of association seen between PEMD and meat *L** in Merino sired lambs and Terminal sired lambs with Merino dams in this study. When myoglobin concentrations were accounted for in our ASBV model, the positive association between PEMD and meat *L** became independent of sire type. Thus the changing effect of sire PEMD on lamb muscle myoglobin concentrations between different sire types may account for the inconsistency of the effect of PEMD on lamb meat *L**. However, accounting for lamb loin myoglobin concentration, iron, IMF or pH_{24} did not account for the positive influence of PEMD on meat *L**. This suggests that selection for PEMD is resulting in other unidentified changes to loin muscle, potentially changes in meat structure that are in turn influencing meat *L**. PEMD was not associated with *L** in those lambs with ICDH measures (n = 2881), thus changes in muscle oxygen consumption rate or fibre type independent of myoglobin and iron concentrations may account for the effect of PEMD on *L** in the total 7731 lambs with ASBV data.

The effects of increasing sire PEMD estimates on lamb loin *a**, *b** and chroma were similarly varied and inconsistent between lambs of different breed types and sexes. Increasing sire PEMD estimates reduced meat *a** and chroma in Terminal sired female lambs from cross bred dams, though unexpectedly increased meat *a** and chroma in Merino sired lambs. This may again relate to the inconsistent effect of sire PEMD on

lamb myoglobin concentrations between different sire types, as reported by Kelman et al. (2014), where an increase in myoglobin concentration in lamb muscle from high PEMD Merino sires would explain the observed improvement in meat *a** and chroma of Merino sired lambs. In line with this the inclusion of lamb myoglobin concentrations did account for the effect of PEMD on meat *a** and chroma in Merino sired lambs. Alternatively changes in IMF accounted for the negative impact of high PEMD on meat *a** and chroma of Terminal sired female lambs from crossbred dams. Thus it is likely that a combination of reduced IMF and muscle oxidative capacity underpins the reduction in meat *a** with increasing PEMD estimates in Terminal sired female lambs with cross bred dams. The lack of association between PEMD estimates and meat *a** and chroma in all lambs from Merino dams may relate to the opposing influences at play with selection for high PEMD in this breed type; reducing IMF and increasing myoglobin concentration.

Increasing sire PEMD estimates increased lamb meat hue angle independent of lamb breed type or sex. Alternatively, sire PEMD was only associated with meat *b** in Maternal sired lambs, where increasing PEMD increased *b**. When IMF, myoglobin concentration or iron concentration were accounted for separately in these models, the positive association between PEMD and *b** became independent of breed type and sex while the effect of sire PEMD on lamb meat hue angle was unchanged. The failure of IMF, muscle oxidative capacity markers or meat pH_{24} to account for the effect of PEMD on meat yellowness measures is surprising, particularly given the strong association between IMF and meat *b** and hue angle measures and the reduction in lamb IMF reported with selection for high PEMD estimates (Pannier, Pethick et al. 2014). The cause of this increased lamb loin yellowness with increasing sire PEMD selection is unclear.

Overall selection for lean meat yield via increasing sire PEMD estimates did have some positive effects on bloomed loin colour. Increasing PEMD was associated with increased meat *L**, *a**, *b**, hue angle and chroma in one or more breed and sex type combinations, however PEMD estimates did not have a significant effect in the majority of lambs, and even reduced meat redness and chroma in one breed type grouping. A combination of reduced myoglobin and IMF concentrations with increasing PEMD appear to account for the effects of PEMD on meat *a** and chroma, however the mechanisms underpinning the associations between PEMD and lamb meat *L**, *b** and hue angle remain unclear.

4.4.3 Effect of sire PFAT estimates on lamb loin *L**, *a**, *b**, hue angle and chroma

In line with our expectations, sire PFAT estimates and meat *L** were negatively associated, meaning that selection for increased lean meat yield via reducing sire PFAT estimates will increase lamb loin *L** on display. Inclusion of muscle myoglobin and/or iron concentration in our model accounted for the negative association between PFAT and *L**; thereby supporting our hypothesis that reduced muscle myoglobin with reducing PFAT outweighs the effect of reduced IMF on lamb meat *L**. This association between PFAT and meat *L** was consistent between all breed type and sex combinations, aligning with Kelman et al. (2014) who reported that reducing sire PFAT estimates had a more consistent and greater magnitude of effect reducing muscle myoglobin concentrations than selection for high sire PEMD estimates.

In contrast, selection for reducing sire PFAT estimates had markedly different associations with meat *a** in lambs from different sire types. This result is surprising given the aforementioned magnitude and consistency of the relationship between sire PFAT estimates and lamb myoglobin concentrations between different breed types

(Kelman, Pannier et al. 2014). Reducing sire PFAT estimates reduced loin meat *a** in all Terminal sired lambs and increased meat *a** in Merino sired (pure Merino) lambs. Incorporating lamb muscle myoglobin concentration in the models accounted for these effects, suggesting that myoglobin concentrations increased resulting in an increased *a** with selection for lower PFAT estimates in pure Merino lambs, contrary to the findings of Kelman et al. (2014). Though having a similar PFAT range for Merino sires, Kelman et al. (2014) used fewer Merino sires than this study (101 versus 161 sires). Thus potentially in this larger data set a different relationship exists between Merino sire PFAT estimates and lamb myoglobin concentration similar to the contrary association between PEMD sire estimates and myoglobin concentration in Merino lambs.

The influence of sire PFAT estimates on meat *b** was also inconsistent and contrasting between lambs of different breed types. Decreasing PFAT estimates was associated with reduced meat *b** in Terminal sired lamb from cross bred dams, which is likely due to a reduction in IMF concentration. In contrast, selection for reducing PFAT estimates increased meat *b** in Maternal sired lambs. Inclusion of pH_{24} in the model accounted for this effect, suggesting that selection for low PFAT estimates may be associated with reduced loin pH_{24} in Maternal sired lambs. Sire PFAT estimates were not associated with lamb meat hue angle in this study, thus the influence of sire PFAT selection on lamb meat yellowness should not be over-interpreted. Lamb meat chroma was positively associated with PFAT in three of the four Terminal sired sex and dam breed combinations, consistent with the effect of PFAT on *a** and *b** in these lambs, and is likely a result of a combination of reduced IMF and myoglobin concentration with selection for lean meat yield using low PFAT sire estimates. This is supported by the fact that the inclusion of myoglobin, iron, or IMF concentration accounted for all sire PFAT effects on meat *b** and chroma.

Overall increasing lean meat yield via selection for reduced sire PFAT estimates has the potential to improve lamb meat *L** on display, though at some expense to meat *a** and chroma. Anecdotally, a reduction in bloomed lamb meat *L** is considered one of the most important problems deterring consumers of lamb meat; thus the potential to increase loin *L** while increasing meat yield with selection for low sire PFAT estimates in all breed types is promising.

4.4.4 Effect of sire PWT estimates on lamb loin *L**, *a**, *b**, hue angle and chroma

Contrary to our hypothesis, increasing lamb lean meat yield via selection for high sire PWT estimates did not impact the colour of their progeny's loin meat. This hypothesis was based on work demonstrating that high PWT sires produce faster growing lambs that are less mature at slaughter (Hall, 2000), have reduced muscle oxidative capacity (Suzuki and Cassens 1983) and thereby lighter meat (Hopkins, Hegarty et al. 2005). However Kelman et al. (2014) reported that increasing sire PWT estimates was associated with increased lamb loin ICDH ACTIVITY, myoglobin concentration and thus muscle oxidative capacity. Regardless of the causative mechanisms, given the reported influences of PWT sires on lamb loin muscle oxidative capacity and myoglobin concentrations it is surprising that sire PWT estimates were not associated with lamb loin *L**, *a**, *b**, hue angle or chroma in this study.

4.4.5 Effect of shortloin muscle weight on lamb loin *L**, *a**, *b**, hue angle and chroma

Increasing shortloin muscle weight proportionate to whole carcass weight had negative effects on lamb loin colour following blooming; reducing meat *L**, *a**, *b**, hue angle and chroma. Shortloin muscle weight may be considered the phenotypic manifestation

of genetic selection for increased PEMD sire estimates. Thereby we would expect an increase in more glycolytic type fibres and reduced muscle oxidative capacity as shortloin muscle weight increases relative to carcass weight. The reduction in meat *a** and chroma with increasing shortloin muscle weight are consistent with a reduction in muscle myoglobin concentration, however the reduction in meat *L**, *b** and hue angle are not.

The reduction in meat *L** with increasing shortloin weight is surprising given that lean meat yield selection using high sire PEMD and low sire PFAT estimates caused an increase in lamb loin *L** in this study. The negative impact of shortloin muscle weight on meat *L** appears to be driven by an associated reduction in loin IMF; as when IMF is accounted for in the model the association between shortloin weight and *L** becomes positive. This suggests the negative influence of reducing IMF is over-riding any positive influence of reducing muscle oxidative capacity on meat *L** with increasing shortloin muscle weight. Similarly the reduction in *b** and hue angle is likely underpinned by a reduction in IMF with increasing shortloin muscle weight. This is supported by the fact that increasing shortloin muscle weight has a positive impact on *b** and hue angle when changes in loin IMF concentrations are accounted for in the model.

A reduction in loin IMF concentration is likely to also be contributing to the reduction in *a** and chroma associated with increasing relative shortloin weight. The negative impact of increasing shortloin muscle weight on *a** and chroma was reduced by around 50% and 30% when changes in IMF were accounted for, supporting that a reduction in IMF and myoglobin are likely driving these effects in combination.

4.4.6 Effect of shortloin fat weight on lamb loin *L**, *a**, *b**, hue angle and chroma

Shortloin fat weight relative to body weight was positively associated with loin meat *L**, *a**, *b**, hue angle and chroma in this study. Changing shortloin fat weight had a consistently greater magnitude of effect on these meat colour parameters than changes in shortloin muscle weight. Increasing shortloin fat weight thus followed the same direction of effect on meat colour as increasing IMF concentration. However industry demands an increase in IMF to improve meat taste coupled with a reduction in shortloin fat to improve lean meat yield. While reducing shortloin fat weight will have negative influences on all aspects of freshly bloomed meat colour, this effect appears to largely be caused by the reduction in IMF associated with reducing shortloin fat weight. When loin IMF concentration was accounted for in the models the effect of shortloin fat weight on *L**, *a** and chroma was reduced by 89%, 38% and 40%, while the effect of shortloin fat weight on *b** and hue angle were reduced by 25%. Thus if selection for reduced shortloin fat weight is accompanied by independent selection for increased IMF then the detrimental effects on fresh meat colour may be minimal.

Interestingly reducing shortloin fat weight and reducing PFAT sire breeding values has opposing effects on meat *L**. This is an unexpected result given that selection for reduced sire PFAT estimates targets a reduction in external fat over the loin region. It appears the influence of shortloin fat weight is more closely related to IMF levels while sire PFAT estimates impact loin meat colour more through changes to muscle oxidative capacity. This is supported by the fact that all associations between PFAT sire estimates and meat colour in this study lose significance with inclusion of myoglobin or iron concentration though remain unchanged with inclusion of shortloin fat weight or IMF concentration.

4.4.7 Comparing magnitudes of effect on bloomed lamb loin colour

IMF concentration had a greater effect on lamb loin *L**, *a**, *b**, hue angle and chroma than the genetic or phenotypic indicators of lean meat yield. These indicators included PEMD, PFAT and PWT (genetic), and shortloin muscle and fat weights corrected for carcass weight (phenotypic). In fact, many of these lean meat yield indicators were impacting colour largely through their associated reduction in IMF, implying that lean meat yield per se is having relatively little direct impact on colour parameters. None-the-less there was variation between the effects of different indicators of lean meat yield. For example, phenotypic indicators (shortloin muscle and fat weight relative to carcass weight) had a greater magnitude of effect on *L**, *a**, *b**, hue angle and chroma than the genetic indicators (PEMD, PFAT or PWT). Shortloin fat weight had a greater magnitude of effect on all bloomed colour parameters than shortloin muscle weight, while PFAT had more consistent effects on loin *L**, *a** and *b** in different breed type and sex combinations than PEMD. These results suggest that increasing lean meat yield via selection for reduced fatness has a greater effect on bloomed lamb loin colour than via selection for increased muscling.

4.5 Conclusion

Increasing IMF content will increase the lightness, redness, chroma and thereby the consumer appeal of bloomed lamb meat. The effect of sire PFAT and PEMD estimates on meat colour varied considerably between lamb breed types while PWT breeding values had no effect on meat colour. Selection for increased lean meat yield using PFAT sire breeding values had more positive than negative effects on lamb loin colour, particularly on meat lightness, while sire PEMD breeding values had no effect on loin colour in the majority of lambs and positive effects in some breed types. The change in

bloomed meat colour with selection for high PEMD and low PFAT sires appear to be driven by associated changes in myoglobin concentration more than by changes in IMF concentration. Some ASBV effects on bloomed meat colour however, such as the positive association between PEMD and meat redness in pure Merinos, could not be accounted for by changes in IMF, myoglobin or any other muscle factor measured including pH_{24}. In these instances it is possible that selection for lean meat yield is changing the morphology of muscle fibres and/or the oxidative metabolic properties of muscle independent of myoglobin concentration and thereby changing parameters of bloomed meat colour. Overall these are important and promising findings for the Australian sheep industry demonstrating that selection for increase lean meat yield will have neutral or positive effects on bloomed meat colour while increasing IMF concentration will not only improve the taste of lamb but will improve the colour of bloomed lamb meat and thereby minimise economic losses associated with consumer rejection of dark or discoloured meat.

Acknowledgements

The authors gratefully acknowledge the financial support of the Cooperative Research Council for Sheep Industry Innovation (Program 3, Next Generation Meat Quality, project 3.1). Considerable staff and resources were provided at each site of the Information Nucleus Flock by the NSW Department of Primary Industries, University of New England, Department of Primary Industries Victoria, SA Research & Development Institute and the Department of Agriculture and Food WA. The authors also wish to thank the many people who assisted with site supervision, sample collection and measurement as well as abattoir staff. For the New South Wales sites; Dr. D. Hopkins, Dr. G. Refshauge, Mr. D. Stanley, Ms. S. Langfield, Mrs. T. Bird-Gardiner, Ms. T. Lamb, Mrs. E. Toohey and Mr. M. Kerr. For the Victorian sites; Dr. E.

Ponnampalam, Mr. W. Brown, Mr. A Naththarampatha and Mr. M. Kerr. For the Western Australian sites; Dr. Kelly Pearce, Mr. K. Hart, Mr. M. Boyce and Mr. A. Williams.

Chapter 5. Factors affecting the colour of lamb meat from the *longissimus* muscle during display: The influence of muscle weight and muscle oxidative capacity

This chapter is published in Meat Science:

Calnan, H.C., Jacob, R.H., Pethick, D.W. and Gardner, G.E. (2014). Factors affecting the colour of lamb meat from the *longissimus* muscle during display: The influence of muscle weight and muscle oxidative capacity. Meat Science. 96: 1049-1057.

Abstract

Spectrophotometric measures were used to determine the redness:browness (R630/R580) of 4238 lamb longissimus muscle after 3 days under simulated display. The results were analysed using linear mixed effects models. Environmental factors represented by effects such as kill group and site of production produced the greatest variation of up to 2.76 units in R630/R580. Isocitrate dehydrogenase activity, reflecting muscle oxidative capacity, reduced R630/R580 by 0.5 units. Selection for high muscling sires increased R630/R580 by 0.27 units, likely due to changes in muscle oxidative capacity. Lamb carcass weight also increased R630/R580 by 0.5 units. Analysis of genotypic factors influencing lamb size and growth rate such as siretype and dambreed further supported that increased growth rate improves meat R630/R580. Our findings suggest that breeding for increased growth rate and increased muscle weight could result in lamb meat retaining its red colour for extended periods whilst on display.

5.1 Introduction

The colour of lamb meat is crucial to ensuring customer appeal and strongly contributes to the value of the product. Consumers associate a brown meat colour with a lack of freshness and quality. Lamb meat currently has a retail shelf life of only 2 days, with product exceeding this display period often discounted or minced, representing a major economic limitation to the Australian lamb industry. With surface browning being the main limiting factor to the shelf life there is a clear need to develop strategies to improve lamb meat colour.

Meat colour changes during display as myoglobin pigments in the meat surface transform upon exposure to oxygen; from primarily purple deoxymyoglobin, to red oxymyoglobin and finally to brown metmyoglobin. Measuring colour at the end of a simulated display is a simple way of describing the stability of a meat's red colour that meat retailers, producers and consumers can relate to. For this study the ratio of reflectance of light at wavelengths 630 nm and 580 nm (R630/R580), previously known as the oxy/met ratio (Hunt et al., 2001), is used to represent the redness:browness of a meat surface. For lamb meat, this parameter tends to approach a minimum value after 3 days of simulated retail display (Jacob, D'Antuono, Gilmour & Warner, (2012) . After 3 days of display lamb meat will have passed through the blooming period (where R630/R580 increases) and the oxidation of myoglobin into metmyoglobin will be well progressed (where R630/R580 decreases). Comparing redness at this time therefore provides an estimation of colour stability albeit nonspecific in relation to metmyoglobin formation and non-chromatic effects. In support of this Khliji, van de Ven, Lamb, Lanza and Hopkins (2010) and Morrissey, Jacob and Pluske (2008) both found a relationship between R630/R580 and consumer perception of meat colour. R630/R580 needs to

exceed 3.3 units for the average consumer to deem lamb meat to have an acceptable red colour (Khliji et al., 2010).

The rate that a meat surface loses its redness is thought to be determined by 1) the rate of oxygen diffusion and consumption, 2) the rate of auto-oxidation of myoglobin pigments into metmyoglobin and 3) the rate of metmyoglobin reducing activity (Faustman and Cassens 1990). Research into meat metmyoglobin reducing activity has produced inconsistent findings and its contribution to meat colour during display remains unclear (Bekhit & Faustman, 2005). Bekhit (2001) found no correlation between metmyoglobin reducing activity and colour stability parameters in lamb loin meat, whereas factors influencing oxygen diffusion and consumption and the auto-oxidation of myoglobin have been shown impact lamb meat colour during display (Jose, 2011). Factors that influence these biochemical processes may be phenotypically associated with economically important traits for lamb production such as growth rate and increased muscle weight.

One important intrinsic muscle factor that could link meat colour to other carcass traits is muscle oxidative capacity. Muscle oxidative capacity refers to the proportion of oxidative type I, oxidative/glycolytic type IIA and glycolytic type IIX myofibres within a muscle. Muscles with higher proportions of oxidative myofibres, such as *m. semimembranosus,* are darker and redder initially with higher myoglobin and iron concentrations. These more oxidative muscles are then prone to more rapid discolouration after slicing for display than the more glycolytic muscle types such as *m. semitendinosus* (O'Keeffe and Hood 1982, Renerre and Labas 1987). King, Shackelford, Rodriguez and Wheeler (2011) demonstrated that the initial oxygen consumption rate and reducing capacity of beef during display contributed to animal variation in colour stability, however the extent to which animal variation in muscle oxidative capacity

changes the colour of a particular muscle during the display of lamb meat is unknown (Gardner, Hopkins et al. 2007, Warner, Ponnampalam et al. 2007).

A relatively simple way of comparing the oxidative capacity of a muscle between different animals is to measure the activity of ICDH. This enzyme is crucial in the oxygen-dependent citric acid cycle of mitochondria, which are larger and more abundant in oxidative myofibres (Hoppeler 1985). Gardner et al. (2007) found a correlation between ICDH activity and myofibre type, allowing measures of ICDH activity to be used as an indicator of muscle oxidative capacity for research purposes that is less difficult to determine and more economical than fibre typing. Furthermore *post-mortem* mitochondrial activity has been linked to retail colour as this influences the oxygen consumption rate of meat (Tang, Faustman et al. 2005).

Variation between animals in the oxidative capacity of a muscle could be influenced by a range of factors such as selection for increased muscle weight. Increased muscling has been shown to increase expression of type IIX glycolytic myofibres (Wegner 2000, Hall, Gilmour et al. 2002, Greenwood, Gardner et al. 2006), and could therefore impact meat colour by effectively changing the myofibre composition of lamb meat. Increased muscle weight in lambs has been achieved in Australia by selecting for sires with a high Australian Sheep Breeding Values (ASBVs) for post-weaning eye muscle depth (PEMD), a value based on ultrasound measurement of the depth of eye muscle adjacent to the 12^{th} rib adjusted for liveweight (Hall, Gilmour et al. 2002, Hall, Gilmour et al. 2002, Hegarty, Hopkins et al. 2006, Gardner, Williams et al. 2010).

Animal maturity has also been shown to alter myofibre composition, with increasing maturity associated with increasing expression of type I oxidative myofibres and thus higher muscle oxidative capacity (White, McGavin et al. 1978, Suzuki and Cassens 1983). The maturity of lambs at slaughter has been reduced in Australia as a result of

increased growth rates (Hall 2000). Lamb growth rates have been increased by selection for sires with high post-weaning weight (PWT) ASBVs (Hall, Gilmour et al. 2002). This breeding value has been associated with larger mature size (Huisman and Brown 2009). Therefore when high PWT lambs are slaughtered at a predetermined weight, they will be younger and less mature at slaughter. Reduced maturity is likely to reduce muscle oxidative capacity which in turn has the potential to impact on the redness of their meat during display

The potential for sire selection based on muscling and growth rate to indirectly change meat colour during display, by altering muscle oxidative capacity, was the key focus of this study. To examine this we measured R630/R580 of the *longissimus lumborum* (loin) muscle after 3 days of simulated retail display and assessed its association with phenotypic carcass measurements relating to lean meat yield, ICDH activity and sire ASBVs for muscling and growth rate. We hypothesised that loin meat derived from lambs with heavier muscle weight would be redder in colour after 3 days of display than meat with high ICDH activity. Hence lamb meat from the progeny of high PEMD sires would have increased R630/R580 on display. Likewise meat from rapidly growing lambs, and lambs from high PWT sires would also have increased R630/R580 on display.

5.2 Materials and Methods

5.2.1 Experimental Design, slaughter and carcass measurement details

Data were collected from 4238 lambs produced in the CRC INF over a 4 year period (2007 - 2010) at multiple sites across Australia (AEC numbers 1-7-02, 1-10-1 and 3-10-13), and has been comprehensively described previously (Fogarty, Banks et al. 2007, van de Werf, Kinghorn et al. 2010). Colour after display was measured from lambs

reared at 5 sites across Australia; Trangie NSW, Cowra NSW, Hamilton VIC, Rutherglen VIC, and Katanning WA (Table 5-1).

The lambs were the progeny of 350 different sires (around 90 sires were used each year across all sites) comprising Terminal sire types (Hampshire Down, Ile De France, Poll Dorset, Southdown, Suffolk, Texel and White Suffolk), Maternal sire types (Bond, Booroola, Border Leicester, Coopworth, Corriedale, Dohne Merino, East Friesian, Prime SAMM and White Dorper) and Merino sire types (Merino and Poll Merino). Semen from all three sire types was used to artificially inseminate Merino dams, while semen from Terminal sires only was used to inseminate crossbred ewes (eg Border Leicester x Merino dams). Hence the lambs were of four sire type and dam breed combinations; Maternal ram and Merino ewe, Merino ram and Merino ewe, Terminal ram and Merino ewe or Terminal ram and crossbred Merino ewe.

The lambs were maintained on extensive pasture grazing, with grain, hay or feedlot pellets supplemented when pasture supply was limited. Further details of breed types used, lamb feeding and management are described elsewhere (van de Werf, Kinghorn et al. 2010, Ponnampalam, Butler et al. 2012). At each site lambs were consigned to smaller groups to be slaughtered on the same day (slaughter groups) to enable the average carcass weight target of 21- 22 kg to be achieved. Given selection for slaughter was made based on weights, the lambs ranged from 134 to 503 days of age at the time of slaughter. Within individual slaughter groups the age of lambs varied by as little as 5 days and by up to 36 days of age. The day prior to slaughter the lambs were yarded, held for 6 hours and then weighed and transported to one of six commercial abattoirs. They were then held in lairage overnight and slaughtered the following day. All carcasses were subjected to medium voltage electrical stimulation (Pearce et al., 2010) and trimmed according to AUS-MEAT specifications (Anonymous, 2005), and then

chilled overnight at 3 - 4 °C before samples were collected. The carcasses had an average deep muscle temperature of 3 °C at the time of sample collection, measured via a probe into the loin muscle at the level of the 12th rib.

All lambs were measured and sampled for a wide range of carcass, meat and growth traits. Carcasses were weighed after slaughter to determine the HCWT of each lamb. At 24 hrs *post-mortem* the entire *m longissimus thoracis (12th rib region) et lumborum (lumbar sacral junction)* or loin muscle and overlaying subcutaneous fat were removed and weighed separately (shortloin muscle weight and shortloin fat weight). To determine intramuscular fat (IMF) a 40g sample of loin muscle was excised from the caudal (lumbar sacral) aspect, all subcutaneous fat and silverskin were removed off the sample before it was diced and stored in 50 ml collection tubes at -20 ºC until subsequent freeze drying using a Cuddon FD 1015 freeze dryer (Cuddon Freeze Dry, NZ). IMF content was then determined using a near infrared procedure in a Technicon Infralyser 450 (19 wavelengths) using the method described by Perry, Shorthose, Ferguson and Thompson (2001) and expressed as a percentage of wet tissue weight. pH measures were taken 24 hrs *post-mortem* (pH_{24}) using a probe inserted into the centre of the loin muscle at the level of the 12th rib, as described by Pearce et al. (2010).

In order to determine ICDH activity 1 g portions of loin muscle were sampled from the carcass within 5 hrs *post-mortem*. The samples were taken above the 12th rib, snap-frozen in liquid nitrogen and stored at -80 °C until ICDH activity was measured according to the method of Briand (1981). After excision of the loin muscle from the carcass at 24 hrs *post-mortem* additional small portions of loin were sampled from the posterior section (at the level of the 12th rib) and frozen for subsequent measurement of myoglobin, iron and zinc concentrations. For myoglobin concentration, a 1g sample of loin muscle was excised, finely diced and stored at -20 °C in a 5ml tube until analysis

using the method of Trout (1991). For iron and zinc, loin samples were frozen at –20 °C, freeze-dried using a Cuddon FD 1015 freeze dryer (Cuddon Freeze Dry, Blenheim, New Zealand) and then approximately 0.2 g dry matter per sample was weighed and prepared according to the USEPA method 200.3 (USEPA 1991). Iron and zinc concentrations were then determined using a Vista AX CCD simultaneous ICP-AES (Varian Australia Pty Ltd).

Table 5-1 Number of lambs at each site with R630/R580 data within year of birth, sex, sire type and dam breed groups.

Site	Year of birth				Sex		Sire type		Dam breed (Terminal sired)		
	2007	2008	2009	2010	M	F	Maternal	Merino	Terminal	Merino	Cross-bred Merino
Trangie 608	0	218	199	191	412	196	120	121	367	147	220
Cowra 702	158	149	199	196	494	208	133	109	460	251	209
Rutherglen 917	290	213	208	206	615	302	137	126	654	116	538
Hamilton 739	183	193	175	188	486	253	129	107	503	305	198
Katanning 1272	392	394	225	261	869	403	303	198	771	664	107
	1023	1167	1006	1042	2876	1362	822	661	2755	1483	1272

5.2.2 Colour sample collection and measurement

After excision at 24 hrs *post-mortem* a full cross section at least 50 mm in length, 50 mm in width and 30 mm in depth was cut from the anterior portion of the loin muscle, posterior to the 12th rib. Each individual sample was then vacuum packaged in clear gas-impermeable plastic (20/80-100 microns, transparent polyamide air impenetrable exterior, polyethylene food approved interior, water vapour transmission rate measured at 23 °C and 85 % R.H – 2,6 gr/mq – 24hr, oxygen permeability measured at 23 °C and 0 % R.H – 50 cm^3/mq – 24hr – bar) and aged in a chiller at 3 – 4 °C for 5 days. After this 5 day ageing period the loin samples were removed from their packaging and re-sliced to a length of 30 mm perpendicular to the long axis of the loin sections. The samples were then placed with the freshly cut meat surface facing upwards on black Styrofoam trays (12 x 12 cm) and wrapped with oxygen-permeable polyvinal chloride film (Resinite "DHW" Meat AEP, 15 µm, oxygen transmission rate of 35650 – 46500 cc/m^2/24hr). The loin meat samples were then placed under display, where conditions of temperature and light have been designed to simulate those commonly encountered in Australian retail stores. The samples were displayed on a flat horizontal surface in a walk-in chiller (3.8 x 3 x 4 m) set to 4 °C with no defrost cycle. The temperature at these settings fluctuated between 2 to 6 °C. An overhead light source was suspended 1.5 m above the samples and consisted of 8 Nelson Fluorescent Meat Display BRB Tubes, 58 W and 1520 mm in length, providing a light intensity of 1000 Lux, as measured with a Dick Smith Electronics Light meter Q1367, with a diffuser fitted to achieve even distribution of light and an actual colour temperature of 4000 K.

After 3 days under these display conditions, light reflectance colour measures were taken of each loin sample through the film within the chiller using a Hunterlab spectrophotometer XE Plus (Cat. No. 6352, model No. 45/0-L, aperture of 3.18 cm).

The light source was set at "D65" with the observer set to 10°. The instrument was calibrated using black glass and white ceramic tiles according to manufacturer directions. The reflectance readings allowed a ratio to be calculated as (% reflectance at 630 nm) / (% reflectance at 580 nm), representing the redness of each sample's surface. 630 nm represents maximal reflection of the red pigment oxymyoglobin whilst 580 nm is the reflectance minimum for pure oxymyoglobin. The other two myoglobin forms that determine surface meat colour, purple deoxymyoglobin and brown metmyoglobin are also reflected at these wavelengths to an extent, with moderate deoxymyoglobin reflection at 630 nm and metmyoglobin being the principle pigment reflected at 580 nm. The ratio R630/R580 thus represents the redness:browness of each meat sample's surface. Two spectrophotometric readings of each sample were taken at 90 degrees on the horizontal plane and the ratios were then averaged for statistical analysis.

5.2.3 Statistical analysis

The R630/R580 data were analysed using linear mixed effects models (SAS Version 9.1, SAS Institute, Cary, NC, USA). The base model tested fixed effects for site, year of birth, sire type, sex within sire type, dam breed within sire type and slaughter group within site by year as well as relevant first order interactions. Sire identification and dam by year identification were included as random terms. Non-significant ($P > 0.05$) terms were removed in a step-wise fashion (see Table 5-3 for final model).

The base model described above was then used to test for associations between R630/R580 and various phenotypic covariates (see Table 5-2). These included HCWT, IMF, pH_{24}, ICDH activity, myoglobin, iron and zinc concentration, and were incorporated one at a time in the base model along with all relevant first order interactions with fixed effects. Non-significant ($P > 0.05$) terms were removed in a stepwise manner. This process was then repeated with each phenotypic covariate also

fitted with HCWT to test whether the observed association was simply a reflection of HCWT. Lastly, shortloin muscle weight and shortloin fat weight were incorporated into the base model (separately) along with HCWT representing the impact of composition (muscling or fatness) on R630/R580. This same approach was taken to test the impact of lamb age at slaughter on R630/R580, with age included as a covariate. However in this case the slaughter group within site by year term was fitted as a random term.

R630/R580 was also tested for associations with sire ASBVs for PWT, PEMD and post-weaning c-site fat depth (PFAT). Initially all 3 ASBVs were included as covariates in the model, as well as their first order interactions with other terms, and non-significant ($P > 0.05$) terms were removed in a stepwise manner. Due to the correlations that exist between these ASBVs in this data set (PWT vs PEMD = 0.3; PWT vs PFAT = 0.3; PEMD vs PFAT = 0.1) this process was repeated with the ASBVs included one at a time to test the independence of their effects. In addition, the effects of the ASBVs were also tested in models corrected for HCWT, shortloin muscle weight, shortloin fat weight, IMF, pH_{24}, ICDH activity, myoglobin, iron, zinc and lamb age. These models contained the ASBVs plus one phenotypic covariate at a time, as well as their first order interactions with other terms. Non-significant ($P > 0.05$) terms were removed in a stepwise manner.

The mean, standard deviation and range for the R630/R580 data, all covariates tested and the ASBVs for each sire type are shown in Table 5-2.

Table 5-2 Descriptive statistics of R630/R580 of the *m. longissimus* after 72 hours of simulated retail display, of the muscle covariates and breeding values analysed.

	Mean	St. Dev	Range
R630/R580	3.05	0.74	1.70 - 8.05
Covariates (units):			
HCWT (kg)	22.7	3.37	12.5 - 36.0
IMF (%)	4.16	1.05	1.50 - 9.83
pH_{24}	5.66	0.14	5.28 - 6.67
Isocitrate dehydrogenase activity (µmol/min/g)	5.18	1.66	1.44 - 11.4
Myoglobin (mg/g muscle)	6.51	1.64	2.15 - 15.6
Iron (mg/100g muscle)	20.0	3.44	8.12 - 45.1
Zinc (mg/100g muscle)	24.0	4.52	11.8 - 44.9
Shortloin muscle weight (g)	354	64.7	157 - 661
Shortloin fat weight (g)	175	79.8	10.0 - 590
Age (days)	251	73.0	134 - 503
Maternal sire ASBV (Australian sheep breeding value) estimates:			
PEMD (post-weaning eye muscle depth)	0.18	0.68	-1.44 - 1.82
PFAT (post-weaning fat depth)	0.04	0.86	-1.62 - 2.56
PWT (post-weaning weight)	5.04	2.57	-3.66 -10.5
Merino sire ASBV estimates:			
PEMD	0.03	1.03	-2.02 - 2.69
PFAT	-0.17	0.71	-1.89 – 1.5
PWT	1.79	3.06	-4.99 – 8.39
Terminal sire ASBV estimates:			
PEMD	1.08	1.20	-2.90 - 4.92
PFAT	-0.82	0.76	-2.44 – 2.27
PWT	12.2	2.47	1.13 – 18.1

5.3 Results

5.3.1 Effects of industry production factors on R630/R580 - base model

The average R630/R580 for all lambs over four years was 3.05 ± 0.01 (± SE) (Table 5-2) and the base model (Table 5-3) described 60% of the variance in redness.

Sites differed markedly in their redness ($P < 0.01$; Table 5-3), with these differences varying each year (Figure 5-1). Generally lambs from Katanning had the highest average R630/R580 across all years (3.21 ± 0.04) compared to Trangie which had the lowest average R630/R580 (2.69 ± 0.04). Less variation was observed between years, with higher R630/R580 recorded in 2007 (3.00 ± 0.04) and 2008 (3.06 ± 0.04), than in 2009 (2.84 ± 0.04) and 2010 (2.82 ± 0.04).

Within each year at each site there were also marked differences in R630/R580 between slaughter groups ($P < 0.01$; Table 5-3), which demonstrated no consistent trend and varied by up to 2.76 units. The average slaughter group R630/R580 ranged from as little as 2.17 units in Rutherglen in 2008 to as high as 4.93 units in Katanning in 2008.

Terminal (3.12 ± 0.02) and Maternal (2.94 ± 0.03) sired lambs had a 0.4 and 0.22 unit higher R630/R580 ($P < 0.01$; Table 5-3) compared to Merino sired lambs (2.72 ± 0.04). Progeny of Border Leicester–Merino dams had a higher R630/R580 (3.22 ± 0.02) than lambs from Merino dams (3.02 ± 0.02) ($P < 0.01$; Table 5-3).

Table 5-3 The F-values, *P*-values and numerator and denominator degrees of freedom for the effects of the base linear mixed effects model of R630/R580 of the *m. longissimus lumborum* of lambs.

Effect	NDF, DDF	F-value
Site	4, 750	70.07*
Year	3, 3065	22.59*
Sire type	2, 750	32.43*
Dam breed (sire type)	1, 750	58.30*
Slaughter group (site*year)	57, 750	45.44*

NDF, DDF: numerator and denominator degrees of freedom; **P-value < 0.01*

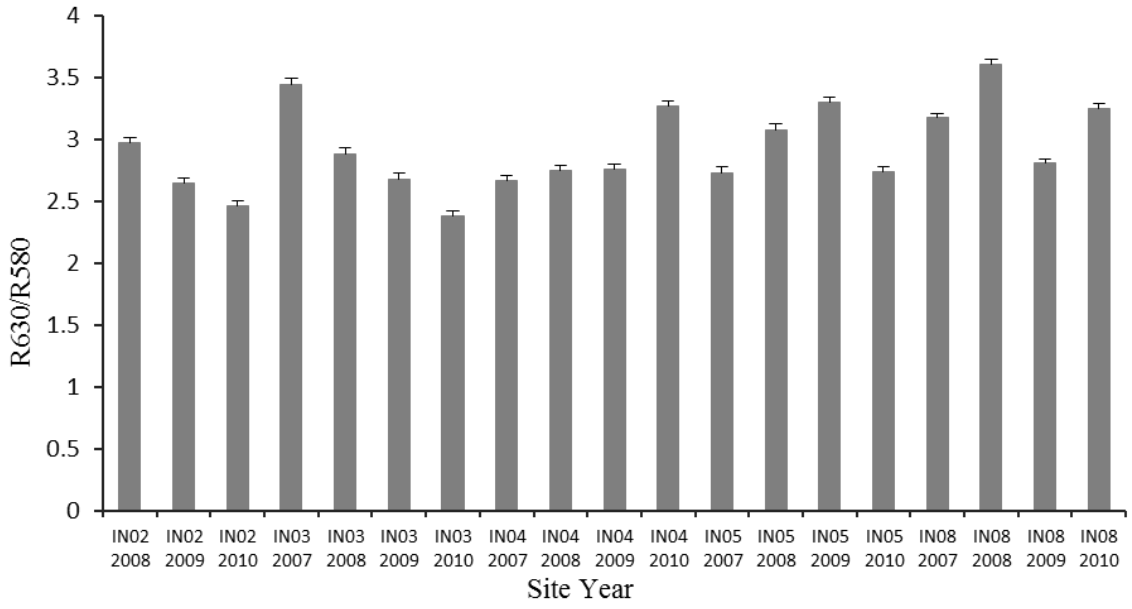

Figure 5-1 Effect of lamb site of production and year of birth on R630/R580.

IN02 represents Trangie NSW, IN03 Cowra NSW, IN04 Rutherglen VIC, IN05 Hamilton VIC and IN08 Katanning WA.

5.3.2. Effect of phenotypic carcass traits on redness

R630/R580 was shown to be improved with higher lamb HCWT ($P < 0.01$). Increasing HCWT across a range of 15 to 30 kg produced a 0.50 unit increase in R630/R580 (Table 5-4). Increasing shortloin muscle weight and shortloin fat weight (separately) also increased ($P < 0.01$) R630/R580 when analysed with HCWT incorporated into the model. Increasing shortloin muscle weight and shortloin fat weight from 100 to 400 g produced a 0.61 and 0.23 unit increase in R630/R580 respectively (Table 5-4). IMF reduced meat R630/R580 ($P < 0.01$), with increasing IMF from 3 to 7% producing a 0.41 unit reduction in redness.

High ICDH activity reduced R630/R580 ($P < 0.01$). Increasing ICDH activity from 3 to 7.5 µmol/min/g of muscle corresponded with a 0.50 unit drop in R630/R580 (Figure 5-3; Table 5-4). This association was not changed when HCWT was included in the model. High iron concentrations also reduced R630/R580 ($P < 0.01$). Increasing iron content from 15 to 35 mg/100g muscle produced a 0.27 unit decrease in redness (Table 5-4), which was reduced to a 0.20 unit change when HCWT was included in the model. In contrast, increasing the myoglobin content from 4 to 12 mg/g of muscle increased R630/R580 ($P < 0.05$) by 0.04 units (Table 5-4). When HCWT was accounted for in the model, high myoglobin then decreased R630/R580 by 0.05 units. Variation in muscle zinc concentration did not have a significant ($P > 0.05$) impact on R630/R580.

High pH_{24} of the loin muscle produced a marked decrease ($P < 0.01$) in R630/R580 after display. Increasing pH_{24} from 5.4 to 6 reduced R630/R580 by 0.88 units (Figure

5-4; Table 5-4). The magnitude of this effect was not altered when HCWT was included in the model. When age at slaughter was added into the model with the slaughter group within site and year term fitted as a random effect, increasing age from 140 to 500 days reduced R630/R580 by 0.61 units ($P < 0.01$; Figure 5-2).

Table 5-4 Effect of covariate phenotypic traits on R630/R580 across their range.

Covariate (unit)	Range	Change in R630/R580	F-value
pH_{24}	5.4 – 6	-0.88	105.85*
Age at slaughter (days)	140 - 500	-0.61	21.71*
Isocitrate dehydrogenase activity (µmol/min/g)	3 - 7.5	-0.5	16.38*
IMF (%)	3 - 7	-0.41^	4.86**
Iron concentration (mg/100g)	15 - 35	-0.27	15.77*
Myoglobin concentration (mg/100g)	4 - 12	0.04	1.42*
Shortloin fat weight (kg)	0.1 - 0.4	0.23^	15.58**
HCWT (kg)	15 - 30	0.5	47.00*
Shortloin muscle weight (kg)	0.1 - 0.4	0.61^	17.03*

^ Represents the effect of a covariate analysed with HCWT included into the model

* Represents $P < 0.01$ whilst ** represents $P < 0.05$

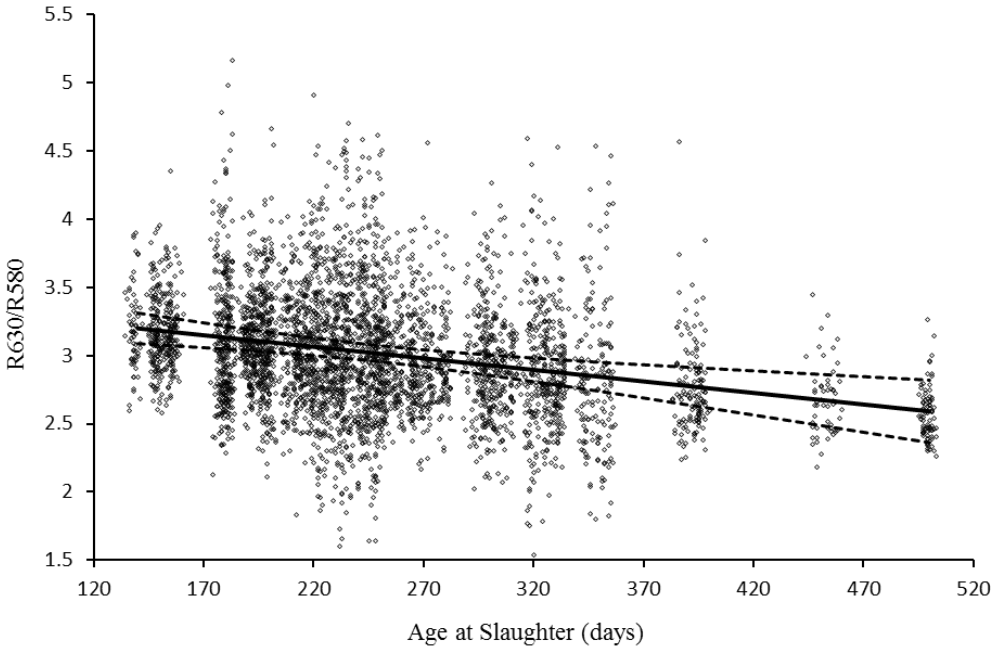

Figure 5-2 The effect of lamb age at slaughter in days on loin R630/R580 after 72 hours of display. Lines represent predicted means for age at slaughter (± SE). Icons represent each residual from the predicted means.

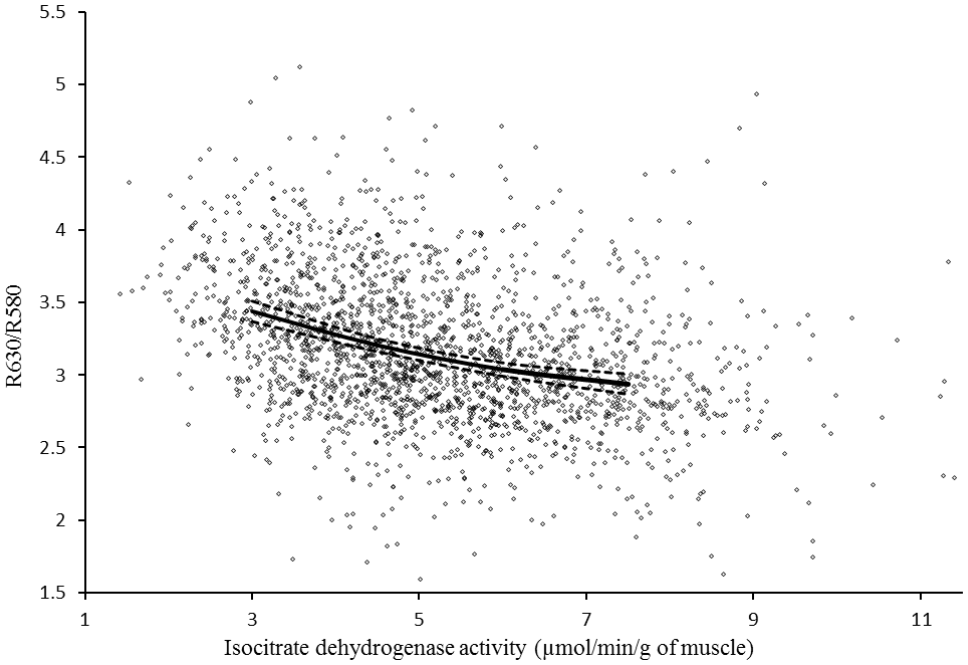

Figure 5-3 The effect of isocitrate dehydrogenase activity on loin R630/R580 after 72 hours of display. Lines represent predicted means ±SE. Icons represent each residual from the predicted means.

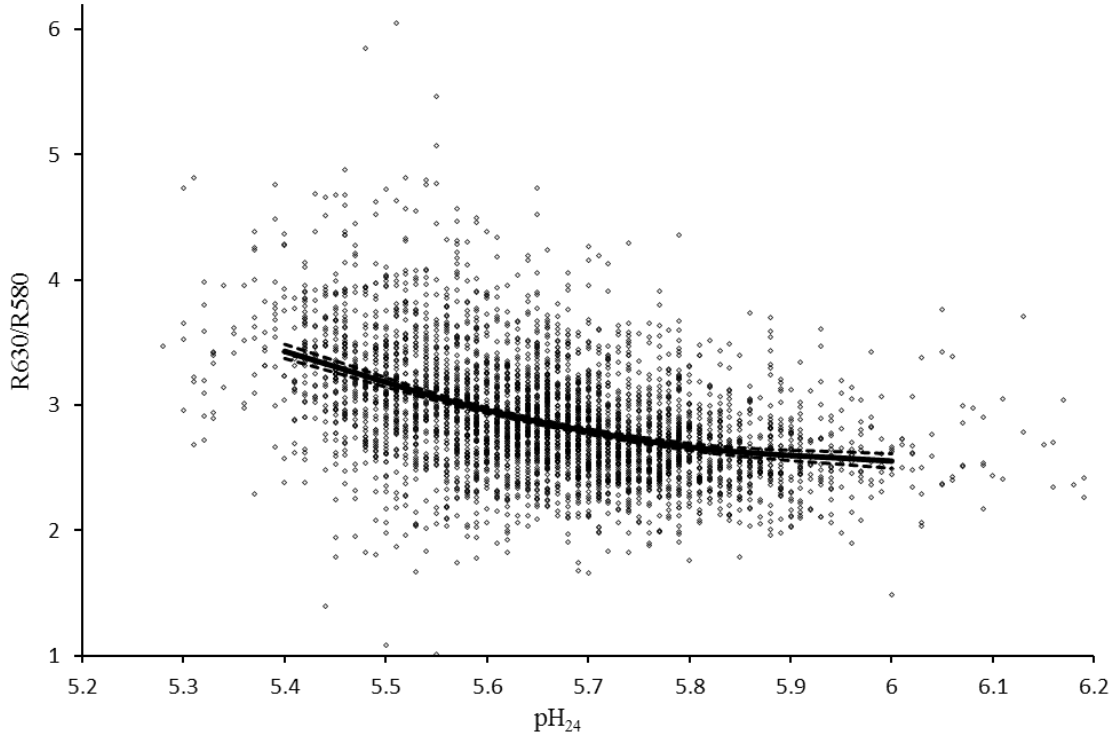

Figure 5-4 The effect of pH_{24} on lamb loin R630/R580 after 72 hours on display.
Lines represent predicted means ±SE. Icons represent each residual from the predicted means.

5.3.3 Effect of sire and sire breeding values on redness

Within the base model sire type had a significant impact on R630/R580 ($P < 0.01$; Table 5-3). The redness of lamb loins produced by individual sires ranged from 2.84 to 3.61 units for Terminal sires, 2.79 to 3.25 units for Maternal sires and 2.62 to 2.85 units for Merino sires (Figure 5-5).

When the sire ASBVs for PWT, PEMD and PFAT were included simultaneously as covariates in the base linear mixed effects model, only PEMD affected ($P < 0.05$)

R630/R580. Increasing PEMD from -2.2 to 4.2 units increased R630/R580 after 72 hours display by 0.27 units (Figure 5-5). When PEMD was incorporated individually in the base model its impact on R630/R580 remained the same. When the phenotypic covariates were included one at a time in the PEMD model, the inclusion of ICDH activity accounted for most of the PEMD effect.

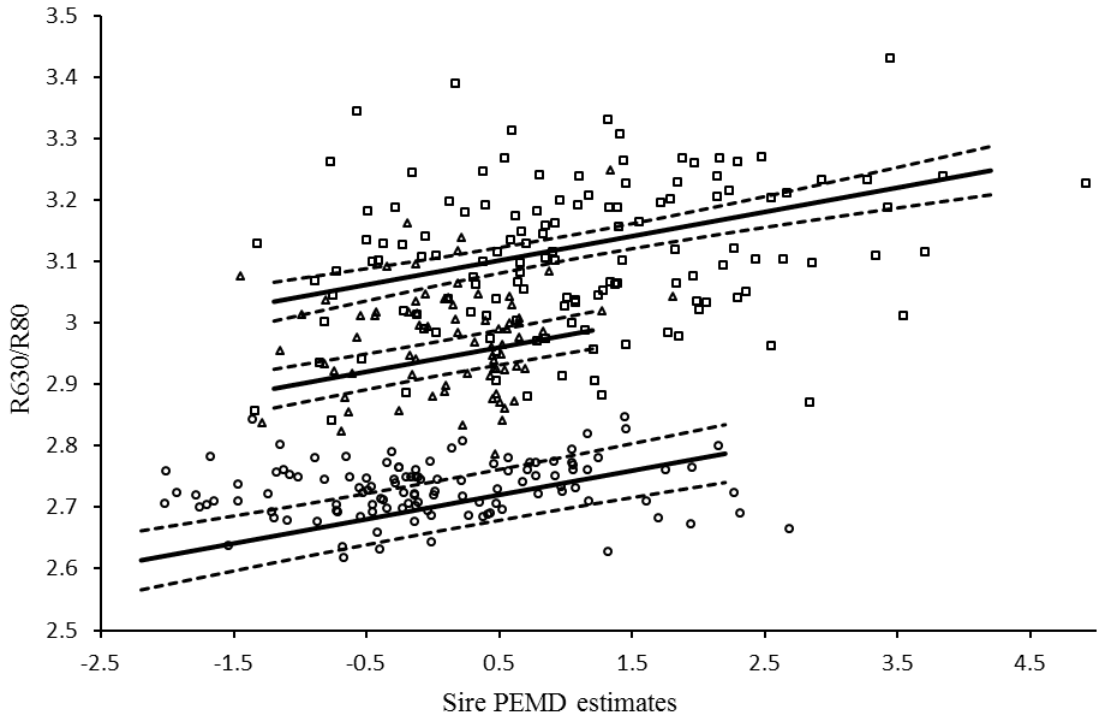

Figure 5-5 The effect of sire Post-weaning eye muscle depth (PEMD) on R630/R580 at 72 hours display. Lines represent predicted means ±SE for each sire type. Icons represent individual sire estimates taken from the base model + sire type predicted mean for: □, Terminal sires; ×, Maternal sires; ○, Merino sires.

5.4 Discussion

In line with our hypothesis, increasing ICDH activity in the loin muscle was associated with a reduction in R630/R580. This supports the notion that colour of lamb meat during display is worsened with increasing muscle oxidative capacity. The importance

of our finding is that the difference was found between lambs in one muscle rather than between different muscles as previously described (O'Keeffe and Hood 1982, Renerre and Labas 1987, Lanari and Cassens 1991, Madhavi and Carpenter 1993). Selection objectives that change the oxidative capacity of muscles such as growth rate and increased muscling could therefore also change the colour of meat on display.

High sire ASBVs for PEMD markedly increased the R630/R580 of lamb meat after 3 days display in this study. This positive relationship supports our hypothesis that selection for muscling would improve meat colour during display. Gardner et al. (2007) demonstrated that selection for muscling reduced oxidative capacity of muscle in sheep, which would lead to the observed improvement in colour. Likewise, Kelman, Pannier, Gardner and Pethick (2013) demonstrated within the same lambs used in the present study that higher PEMD breeding values were associated with reduced ICDH activity. Thus it appears that the increase in meat R630/R580 by 0.27 units seen with high PEMD breeding values is delivered via its correlated reduction in muscle oxidative capacity. This notion is further supported because PEMD had a reduced impact on R630/R580 when the model was corrected for ICDH activity. These findings suggest that selecting sires for muscling using the ASBV for PEMD will cause a relative increase in the R630/R580 of their progeny's meat whilst on display by up to 24 hours (Jacob et al., 2013). Given 3 days is the end of the display period used in domestic markets, selecting for muscling should improve the shelf life of lamb meat in this context.

In this study increased HCWT represents increased growth rate, given that our model was corrected for slaughter group and lambs within slaughter groups were of similar

ages. Thus the positive association between HCWT/growth rate and R630/R580 supported our hypothesis. Alternatively, increasing growth rate via selection of sires with high PWT estimates did not have an impact on the colour of lamb loins after display, contrary to our hypothesis. The basis both of these hypotheses was that faster growth rates were likely reflective of genotypes with a larger mature size. Therefore faster growing lambs when compared at the same age would be less mature and their muscle less oxidative (Brandstetter, Picard et al. 1998), resulting in improved meat colour. While increasing PWT has been linked with increased mature size (Huisman and Brown, 2009), its lack of impact on R630/R580 casts some doubt on the fibre type/maturity linked premise of our hypothesis. Likewise the phenotypic variation in HCWT may not necessarily imply variation in maturity and subsequent altered fibre type. This is further supported by the fact that there was no change to the association between R630/R580 and HCWT when we corrected the model for ICDH activity. Finally, Kelman et al. (2013) demonstrated no consistent association between ICDH and HCWT within these same lambs. Therefore it appears that the impact of phenotypic growth rate on meat colour is not delivered via altered muscle oxidative capacity, but perhaps by another alteration to muscle metabolism that impacts myoglobin oxidation or reduction processes.

The progeny of Terminal and Maternal sires had better meat colour than those of Merino sires. Likewise, progeny from Border-Leicester-Merino dams had better colour than those from pure Merino dams. These results align well with our hypotheses regarding selection for muscling, with the greater muscling potential of the Terminal and Maternal genotype lambs when compared to Merino lambs likely to have impacted

muscle fibre type reducing oxidative capacity (Hawkins, Moody et al. 1985, Greenwood, Harden et al. 2007). This assertion was supported by correcting the model for ICDH activity which accounted for these genotype differences. This result is unlikely to reflect variation in age. Although Merino lambs are slower growing and were therefore often grouped together in later slaughter groups, there were a number of slaughter groups (n = 55) where Terminal and Maternal lambs were slaughtered together with Merino lambs enabling the direct comparison of these sire types without confounding with age.

Importantly, in this study meat R630/R580 after 3 days display was more strongly associated with ICDH activity than iron or myoglobin concentrations. While lower ICDH activity was associated with a marked increase in R630/R580, the association with R630/R580 for iron was only half this magnitude across its range, and almost non-existent for myoglobin. This supports the work of Mortimer et al. (2013) that found a phenotypic correlation of -0.12 between meat R630/R580 and ICDH activity, compared to a correlation of only 0.04 and -0.02 with myoglobin and iron concentrations respectively. In this study high iron concentration reduced meat R630/R580 by 0.27 units, supporting that more oxidative muscles brown more rapidly, given that higher iron levels are found in more oxidative muscles (Lefaucheur 2010). In contrast, the small positive impact of myoglobin on R630/R580 by 0.04 units was unexpected given that myoglobin is also more concentrated in more oxidative muscles (Lefaucheur 2010). An explanation for this may be the positive association demonstrated by Kelman et al. (2012) between myoglobin and HCWT. Within this study growth rate is reflected by HCWT at the same slaughter age (ie model corrected for slaughter group) and was

positively associated with R630/R580 of meat colour. Thus in the context of meat colour, myoglobin content appears to be more strongly reflecting variation in growth rate than variation in muscle oxidative capacity. In support of this assertion high myoglobin reduced R630/R580 by 0.05 units when the model was corrected for HCWT.

Increasing pH_{24} of loin muscle also had a strong impact on meat colour, dramatically reducing the R630/R580 across the pH_{24} range. Mortimer et al. (2013) also found negative phenotypic (-0.16) and genetic (-0.37) correlations between meat colour and pH_{24}. The exact mechanisms underpinning this relationship have been poorly defined, but may relate to the impact of pH_{24} on oxygen exchange at the meat surface. A high pH_{24} reduces the amount of oxygen able to penetrate through the meat surface due to water binding and swelling of proteins (Lawrie 1958). Additionally, a high pH_{24} enhances utilization of oxygen by mitochondria, with both factors resulting in less oxygen available to form the red pigment oxymyoglobin (Lawrie 1958). These results are consistent with Jacob et al. (2013), who also identified pH as a key carcass trait needing to be minimised to prevent detrimental effects on lamb meat colour during display.

IMF content reduced the R630/R580 of the lamb loin muscle after display. This supports the phenotypic (-0.14) and genetic (-0.31) correlations reported by Mortimer et al. (2013). Studies have demonstrated a synergistic relationship exists between lipid and myoglobin oxidation (Faustman, Sun et al. 2010), though it is not known whether extracellular lipid peroxidation could promote intra-fibre myoglobin oxidation. Alternatively, significant correlations have been found between IMF and intracellular lipid content (Essén-Gustavsson, Karlsson et al. 1994). Thus increasing IMF could

reflect an associated increase in intracellular lipid, where lipid peroxidation would more directly impact on myoglobin oxidation. It is also ambiguous whether the close relationship between IMF and meat colour relates to the dependence of both processes on similar optimal conditions and substrates such as oxygen and iron, or whether myoglobin oxidation is directly promoted by primary and secondary reactive products of lipid oxidation (Faustman, Sun et al. 2010). These results point out the need for careful management of IMF and colour. For example IMF is a positive consumer trait since it improves tenderness and eating quality (Mortimer, van der Werf et al. 2012, Pannier, Pethick et al. 2012) while on the other hand IMF potentially reduces the R630/R580 of meat during display.

The influence of lamb age on meat colour is difficult to differentiate from a slaughter day effect as it is confounded by slaughter group within our data-set. So whilst we cannot completely discount the possibility of a biased estimate for the magnitude of age, the age associated trend (Figure 5-2) was strongly evident. The 0.61 unit reduction in R630/R580 seen over the increasing age range is likely underpinned by increased muscle oxidative capacity associated with increasing maturity.

There was significant variation in meat colour in this study between lambs produced at different sites, over different years and between different slasughter groups. Given the great variation in geographic regions and climates between sites, we anticipate great variation in factors such as nutrition and the age of lambs reaching slaughter between different production sites and years. With nutrition and age both influencing a lamb's muscle oxidative capacity and growth rate (Moody, Kemp et al. 1980, Brandstetter, Picard et al. 1998), these factors could account for some of the variation evident

between sites and years. However, correction for growth rate, muscle oxidative capacity and age via inclusion of HCWT, ICDH activity and slaughter group terms in the model demonstrated that these factors only account for about 20% of the variation between sites and years. This demonstrates that there is more to nutrition than just its impact on muscle oxidative capacity and growth rate, with changes in antioxidant status likely to be central to the observed changes in meat colour.

Previous work by Khliji et al. (2010) has demonstrated that R630/R580 values equal to or above 3.3 units are required for customer acceptance of meat. In this study the average R630/R580 measured in lamb loins after 3 days of simulated retail display failed to reach this level, with approximately 70% of the lambs measured falling under 3.3 units. Yet importantly, this work has demonstrated significant variation in meat colour and identified some of the key factors influencing it. Different slaughter groups from the same site within the same year created the largest magnitude change in R630/R580 of up to 2.56 units, while there was up to 0.52 units difference between sites over the four year period and 0.24 units difference observed between years at any given site. The phenotypic carcass traits with the largest impact on R630/R580 were pH_{24} and shortloin muscle weight, producing a 0.88 unit reduction and 0.61 unit increase in redness respectively. ICDH activity, lamb age at slaughter and HCWT each accounted for approximately 0.50 units change in R630/R580. There was a 0.40 unit difference between sire types and a 0.20 unit difference between dam breeds. The ASBV for PEMD produced a 0.27 unit change in R630/R580, while there was a 1.0 unit difference in R630/R580 between sire estimates for different individual sires.

5.5 Conclusion

Aligning with our hypotheses, this comprehensive study supported general thinking that muscle oxidative capacity is one of the key drivers of lamb meat colour on display. Selection for increased muscling improved the R630/R580 of meat after display, likely driven by changes in muscle oxidative capacity. Increasing carcass weight, representative of growth rate, also increased meat R630/R580, though independent of muscle oxidative capacity. Despite environmental factors such as slaughter group accounting for more of the observed variation in meat colour, the genotypic effects demonstrated are comparatively large and suggest the potential to produce more lamb meat of an acceptable colour at 3 to 4 days of display. The potential for genetic manipulation is supported by the work of Mortimer et al. (2012) that found the R630/R580 after 2 days of display to have correlation coefficients of 0.34 for phenotype, 0.10 for genotype and a heritability of 0.30 (± 0.04). While the potential to develop a sire estimated breeding value encompassing retail meat colour is promising, establishing the interactions between carcass traits and colour is also important for industry prediction and monitoring purposes; in order to ensure that meat redness and thus shelf life are not inadvertently reduced with selection pressures for different meat carcass traits such as IMF or lean meat yield.

Acknowledgements

The authors gratefully acknowledge the financial support of the Cooperative Research Council for Sheep Industry Innovation. Considerable staff and resources were provided

at each site of the Information Nucleus Flock by the NSW Department of Primary Industries, University of New England, Department of Primary Industries Victoria, SA Research & Development Institute and the Department of Agriculture and Food WA. The authors also wish to thank the people who assisted with site supervision, sample collection and measurement as well as abattoir staff. For the New South Wales sites; Dr. D. Hopkins, Dr. G. Refshauge, Mr. D. Stanley, Ms. S. Langfield, Mrs. T. Bird-Gardiner, Ms. T. Lamb, Mrs. E. Toohey and Mr. M. Kerr. For the Victorian sites; Dr. E. Ponnampalam, Mr.W. Brown, Mr. A Naththarampatha and Mr. M. Kerr. For the Western Australian sites; Dr. Kelly Pearce, Mr. K. Hart, Mr. M. Boyce and Mr. A. Williams.

Chapter 6. Initial bloom colour is a poor predictor of colour stability in lamb *longissimus* meat

Abstract

The ability of meat colour at the start of retail display to predict meat browning over subsequent retail display was investigated in lamb loin meat. Mixed breed lambs (n = 4404) were slaughtered at ~23kg carcass weight and measured for loin pH, intramuscular fat and retail colour. Colour samples were aged in vacuum-packaging for 5 days before being re-sliced, overwrapped and placed under simulated retail display for 72 hours. Meat redness (R630/R580) was measured 24 hourly across display using a Hunterlab spectrophotometer. Simple and partial correlation coefficients between initial and subsequent R630/R580 measures were ≤ 0.4 despite incorporation of carcass traits such as pH, demonstrating that bloomed colour at the start of display is not a useful predictor of meat browning after 48 to 72 hours of retail display. Correlations between 24, 48 and 72 hour measures were high (> 0.8), suggesting that meat colour measured from 24 hours of display can provide an accurate prediction of subsequent browning.

6.1 Introduction

The rapid change in lamb meat colour from red to brown during retail display is an important factor limiting its marketability (Jeyamkondan, Jayas et al. 2000). Meat colour is critical to consumer appeal - consumers expect lamb meat to have a bright red

colour, and associate brown meat with reduced meat quality, a lack of freshness and imminent spoilage. Surface browning will thus deter a consumers' purchase of lamb meat. The poor colour stability of overwrapped lamb meat causes retailers to discount product after about 2 days on display to ensure sale prior to the appearance of browning. Alternatively, a shelf life of up to 8 days can be achieved with high-oxygen modified atmosphere packaging (Channon, Baud et al. 2005), though this packaging reduces sensory appeal of lamb meat (Geesink, Robertson et al. 2015). Substantial variation remains between different animals in their meat colour stability (Calnan, Jacob et al. 2014, Jacob, D'Antuono et al. 2014), thus a simple and practical method to predict the colour of lamb meat on display would help retailers to maximise display time of lamb meat and minimise the amount down-graded.

Lamb meat forms a bright red colour with blooming as oxygen binds with myoglobin to form the red pigment oxymyoglobin. With time after blooming, *post-mortem* metabolism depletes oxygen concentrations and generates free-radical species in meat, which both favour the oxidation of myoglobin into the brown pigment metmyoglobin (Hood and Riordan 1973). *Post-mortem* oxidative metabolism influences the depth of penetration and availability of oxygen to bind with myoglobin during blooming as well as the subsequent oxidation of myoglobin (Atkinson and Follett 1973, Monin and Ouali 1992). Muscle oxidative metabolism therefore influences both the bloomed colour of lamb meat and its colour stability over retail display, and it would be reasonable to expect that colour stability may be associated with bloomed meat colour at the start of retail display. If this association between bloomed meat colour and its subsequent browning on retail display could be demonstrated, measures of bloomed meat colour

early in the *post-mortem* period may be used to predict meat colour stability without the need to expose meat to a simulated display period.

Accounting for muscle factors such as ultimate meat pH (pHu), oxidative capacity and intramuscular fat (IMF) that influence the colour stability of lamb meat (Calnan, Jacob et al. 2014) may improve the ability of bloomed meat colour to predict colour stability on retail display. However these factors are not currently measured or controlled in industry. High pHu, muscle oxidative capacity and IMF have been associated with increased rates of browning in lamb meat on retail display (Atkinson and Follett 1973, Hood 1980, Calnan, Jacob et al. 2014). High pHu is associated with increased metabolic activity and thereby the production of oxidative free-radicals that cause myoglobin oxidation (Faustman and Cassens 1990). Ultimate pH can be approximated in lamb carcasses by measuring pH at 24 hours *post-mortem* (pH_{24}). Similarly, muscle with higher oxidative capacity browns at a faster rate due to higher free-radical production (O'Keeffe and Hood 1982, Renerre and Labas 1987). Muscle oxidative capacity is impractical to measure in an industry setting, however lamb age may be a useful predictor of muscle oxidative capacity given that lamb muscles become increasingly oxidative with age or maturity (Kelman, Pannier et al. 2014). The IMF content of lamb muscle increases lamb meat browning (Renerre and Labas 1987, Calnan, Jacob et al. 2014) via lipid peroxidation, which also causes free-radical production and increases metmyoglobin formation (Faustman, Sun et al. 2010). Measuring IMF is not currently practicable in the lamb industry, despite the value of this measure to predict eating quality and potentially to predict meat browning on display (Thompson 2004). However, technology to measure IMF is rapidly advancing and may soon be available

to industry. Therefore there is the potential for retailers to use information on meat pH_{24}, lamb age and IMF content to improve predictions of lamb meat browning.

The extent of meat browning may be inferred using spectrophotometric light reflectance to measure the redness or red: brown of a meat surface (R630/R580). These ratios progressively reduce as lamb meat browns over retail display, where R630/R580 values below 3.3 units will lead to consumer rejection of lamb meat based on its colour (Khliji et al, 2010). The objective of this study is to determine if knowing the lamb age, pH_{24} and IMF concentration of lamb meat in addition to measuring bloomed meat redness (R630/R580) at the start of retail display may provide enough information for retailers to predict the rate of meat browning on subsequent retail display. Retailers could then predict when the threshold for colour acceptability may be reached and maximise the retail display period of premium valued meat. We hypothesise that knowledge of lamb age, pH_{24}, IMF concentration and surface R630/R580 at the start of retail display will allow the prediction of lamb loin redness (R630/R580) at the end of a 72 hour simulated retail display period.

6.2 Material and methods

6.2.1 Experimental design

The Sheep CRC produced 4404 lambs at multiple sites across Australia between 2007 and 2011 as part of the INF experiment (AEC numbers 1-7-02, 1-10-1 and 3-10-13). The design of the INF has been comprehensively described previously (Fogarty, Banks et al. 2007, van de Werf, Kinghorn et al. 2010). Meat colour across a simulated retail

display was measured in lambs reared at 5 production sites across Australia: Cowra and Trangie in New South Wales, Hamilton and Rutherglen in Victoria, and Katanning in Western Australia. Climatic conditions vary substantially between these sites which are up to 3400 km apart.

Industry-proven sires were selected for artificial insemination mating with the ewe flocks located at each site. A total of 451 sires were selected from a range of breeds used in the Australian sheep industry (Merino, Maternal and Terminal breed types). Around 90 sires were used each year across all sites of production. Terminal sire types included Hampshire Down, Ile De France, Poll Dorset, Southdown, Suffolk, Texel and White Suffolk breeds, while Maternal sire types included Bond, Booroola, Border Leicester, Coopworth, Corriedale, Dohne Merino, East Friesian, Prime SAMM and White Dorper breeds and Merino sire types included Merino and Poll Merino breeds. Merino dams were inseminated with semen from all three sire types, while crossbred ewes (eg Border Leicester x Merino dams) were inseminated only with semen from Terminal sires. Very few female Maternal and Merino sired lambs were sent to slaughter as they were retained for breeding purposes. Thus effective comparisons between lamb sexes can only be made within the Terminal sired lamb groups.

The lambs were thus of six sire type, dam breed and sex combinations in this study; Maternal sired male progeny of Merino dams, Merino sired male progeny of Merino dams, Terminal sired male progeny of Merino dams, Terminal sired female progeny of Merino dams, Terminal sired male progeny of crossbred Merino dams and Terminal sired female progeny of crossbred Merino dams. The lambs were generally maintained on extensive pasture grazing, though grain, hay or feedlot pellets were supplemented

when pasture supply was limited. Further details of lamb management, nutrition and genetic design of this experiment have been previously published (van de Werf, Kinghorn et al. 2010, Ponnampalam, Butler et al. 2012).

6.2.2 Slaughter details

At each site lambs were consigned to smaller groups to be killed on the same day (slaughter groups). Selection of lambs for slaughter groups was based on their live weight measures with the aim to achieve an average carcass weight of 20 - 25 kg. There were a total of 73 slaughter groups containing an average of 60 lambs in each, though the number of lambs per slaughter group ranged from 23 to 131. Each site produced 2 to 5 slaughter groups of lambs each year, most commonly 3 to 4. The time of year that lambs were sent to slaughter varied considerably between slaughter groups, between sites and years of production.

Given selection for slaughter was made based on live weights, there was a large range in the age of lambs between slaughter groups. Lambs were an average of 8 months old (or 248 days) at the time of slaughter, though ranged in age from 134 to 503 days at slaughter. However given lambs were bred via artificial insemination and thus were typically born within a short period of time, the age range of lambs within slaughter groups was comparatively small. The age range of lambs within slaughter groups was 11 days on average, varying by as little as 5 days and by up to 36 days difference in age.

The day prior to slaughter the lambs were yarded, held for 6 hours and then weighed and transported to a commercial abattoir. The trucking distance required to deliver slaughter groups to the abattoir varied considerably, though were consistent for each site

of production in this study. At the abattoir, lambs were held in lairage overnight before slaughter the following day. Lamb carcasses were electrically stimulated on medium voltage (Pearce, van de Ven et al. 2010) and trimmed according to AUS-MEAT specifications (Anonymous 1992), before chilling overnight at 3 - 4 °C.

6.2.3 Carcass and muscle sampling

All lambs were measured and sampled for a wide range of carcass, meat and growth traits. The HCWT (kg) of each lamb was measured on the chain following slaughter. A small portion of loin muscle was collected adjacent to the 12^{th} rib to measure ICDH activity as soon as possible (a maximum of 5 hours) *post-mortem*. A 1 g portion of loin muscle was snap-frozen in liquid nitrogen and stored at - 80 °C until ICDH could be measured according to the method of Briand (1981).

Lamb carcasses from each slaughter group were hung together in a chiller at 3 - 4 °C for between 20 and 25 hours before the entire *m. longissimus thoracis (*12^{th} *rib region) et lumborum (lumbar sacral junction)* or shortloin muscle and overlaying subcutaneous shortloin fat were removed. These components were weighed separately to determine the shortloin muscle weight (g) and shortloin fat weight (g). A TPS WP-80 pH and temperature meter (with a Mettler Toledo puncture pH probe- LoT406-M6-DXK-S7/25) was calibrated at pH 4 and 7 within the chiller before being used to measure loin muscle temperature and pH (pH_{24}) via the insertion of the probe into the centre of the loin muscle adjacent to the 12^{th} rib, as further described by Pearce et al. (2010). The carcasses had an average muscle temperature of 3.6 °C (±1.9) at this time.

A 40g muscle sample was excised from the caudal (lumbar sacral) aspect of the loin muscle to determine IMF concentration. All subcutaneous fat and silverskin were removed before the sample was diced and stored in 50 ml collection tubes at -20 ºC until freeze drying using a Cuddon FD 1015 freeze dryer (Cuddon Freeze Dry, NZ). IMF concentration was then determined using a near infrared procedure in a Technicon Infralyser 450 (19 wavelengths) via the method described by Perry, Shorthose, Ferguson and Thompson (2001) and was expressed as a percentage of wet tissue weight.

Additional small portions of the excised loin muscle were sampled from the posterior section (at the level of the 12th rib) and frozen for subsequent measurement of myoglobin, iron and zinc concentration. To determine the myoglobin concentration of the loin muscle a 1g sample was excised, finely diced and stored at -20 °C in a 5ml tube until analysis using the method of Trout (1991). For iron and zinc, loin samples were frozen at –20 °C, freeze-dried using a Cuddon FD 1015 freeze dryer (Cuddon Freeze Dry, Blenheim, New Zealand) and then approximately 0.2 g dry matter per sample was weighed and prepared according to the USEPA method 200.3 (USEPA 1991). Iron and zinc concentrations were then determined using a Vista AX CCD simultaneous ICP-AES (Varian Australia Pty Ltd).

6.2.4 Colour sample collection and measurement

A section of loin muscle was retained from each lamb for measurement of meat colour over a 72 hour simulated retail display. After excision of the entire loin muscle from the carcass at 24 hrs *post-mortem*, a full cross section at least 50 mm in length, 50 mm in

width and 30 mm in depth was cut from the anterior aspect of the loin muscle, posterior to the 12th rib. Each muscle sample was then individually vacuum packaged in clear gas-impermeable plastic (20/80-100 microns, transparent polyamide air impenetrable exterior, polyethylene food approved interior, water vapour transmission rate measured at 23 °C and 85 % R.H – 2,6 gr/mq – 24hr, oxygen permeability measured at 23 °C and 0 % R.H – 50 cm^3/mq – 24hr – bar) and stored in a dark chiller at 3 – 4 °C for 5 days.

Following the 5 days of anoxic storage the loin samples were removed from their vacuum packaging and re-sliced to create a fresh meat surface. The samples were sliced to a length of 30 mm perpendicular to the long axis of the loin muscle. The freshly sliced loin muscle samples were then repackaged on black Styrofoam trays (12 x 12 cm) and wrapped with oxygen-permeable polyvinal chloride film (Resinite "DHW" Meat AEP, 15 µm, oxygen transmission rate of 35650 – 46500 cc/m^2/24hr). The freshly cut meat surface was facing upwards and in full contact with the overwrapped film.

Meat samples were displayed on a flat horizontal surface in a walk-in chiller (3.8 x 3 x 4 m) set to 4 °C with no defrost cycle. The temperature within the chiller fluctuated between 2 to 6 °C on these settings. An overhead light source was suspended 1.5 m above the samples and consisted of 8 Nelson Fluorescent Meat Display BRB Tubes, 58 W and 1520 mm in length, providing a light intensity of 1000 Lux, as measured with a Dick Smith Electronics Light meter Q1367. A diffuser was fitted to the lights to achieve an even distribution of light and an actual colour temperature of 4000 K.

The surface colour of each loin sample was measured 24 hourly over the simulated retail display. The first measurement was taken 30 minutes after slicing to allow for the

cut surface to “bloom”. A Hunterlab spectrophotometer XE Plus (Cat. No. 6352, model No. 45/0-L, aperture of 3.18 cm) was used to measure light reflectance. This was done in the chiller with the spectrophotometer aperture flush with the overwrapped meat surface. The light source of the Hunterlab was set at “D65” with the observer set to 10°. The instrument was calibrated using black glass and white ceramic tiles according to manufacturer directions. From the reflectance values meat redness was calculated defined as the % reflectance at 630 nm / % reflectance at 580 nm (R630/R580) (AMSA 2012). Two spectrophotometric measures were taken of each sample at 90 degrees on the horizontal plane and the ratios were then averaged for statistical analysis.

6.2.5 Statistical analysis

Correlations between R630/R580 values at 0, 24, 48 and 72 hours of simulated retail display were analysed in SAS (SAS Version 9.1, SAS Institute, Cary, NC, USA), with simple correlations estimated using the PROC CORR command, and partial correlations estimated using a multivariate analysis (Table 6-2). In the multivariate analysis the correlations between R630/R580 values at different time points were corrected for fixed effects including site, year, slaughter group within site by year, and sex and dam breed within sire type. The same data set was used to calculate simple and partial correlations. The correlations between R630/R580 time point measures were then tested in a multivariate analysis with different phenotypic covariates (pH_{24}, myoglobin, iron, ICDH activity, zinc, IMF, HCWT, shortloin muscle and fat weight, lamb age) accounted for in the model. Each phenotypic covariate was tested separately in each model, along with their squared term and interactions with fixed effects, before non-significant terms were removed ($P > 0.05$). Lamb age, pH24, IMF were also incorporated together, along with

their interactions with fixed effects, while shortloin muscle and fat weight were analysed with HCWT incorporated to represent the impact of relative composition (muscling or fatness).

R630/R580 values at each time point (0, 24, 48 and 72 hours) were then analysed separately using linear mixed effects models. Fixed effects for site, year, slaughter group within site by year, sex and dam breed within sire type were included in these models along with random terms for sire and dam by year. Non-significant ($P > 0.05$) terms were removed in a step-wise fashion to form a base model for each time point R630/R580 (Table 6-3).

These models were then used to test the association between the phenotypic carcass covariates and each R630/R580 measure over simulated retail display. The phenotypic covariates were incorporated one at a time into the linear mixed effects models for R630/R580 at 0, 24, 48 and 72 hours display, along with the squared term covariate, any interactions with fixed effects and random terms. Non-significant terms ($P > 0.05$) were removed to form a final model that was used to predict the mean R630/R580 across the covariate range (Figure 6-1). Shortloin muscle and fat weight were again analysed in conjunction with HCWT. The impact of lamb age at slaughter on R630/R580 was also regressed in a linear mixed effects model, however given the small variation in lamb age within slaughter groups and substantial variation in age between different slaughter groups, slaughter group within site by year term was fitted as a random term.

Linear mixed effects models were then used to assess the associations between R630/R580 values at different time points of simulated retail display (Figure 6-2). In

these models fixed terms for production site, year and slaughter group within site by year were not included, given that this information is very unlikely to be known to a retailer interested in predicting lamb meat browning. R630/R580 at 0 hours was incorporated as a covariate into the base models for R630/R580 at 24 and 72 hours, along with its squared term and any interactions with sex and dam breed within sire type, with the same random effects and process of step-wise removal of non-significant effects ($P > 0.05$). The same process was used to incorporate R630/R580 at 24 and 48 hours separately as covariates into the model of R630/R580 at 72 hours. These models were used to determine the coefficient of determination (R-Square) and root mean square error for each association (Figure 6-2, Table 6-4).

These same models were then used to test the influence of phenotypic covariates (pH_{24}, myoglobin, iron, ICDH activity, zinc, IMF, HCWT, shortloin muscle and fat weight, lamb age) on the associations between R630/R580 at different time points (Table 6-4). The covariates were incorporated one at a time into each model along with their squared term and interaction with sex and dam breed within sire type. Non-significant ($P > 0.05$) terms were removed. This process was then repeated with each phenotypic covariate also fitted with HCWT to test whether the observed association was simply a reflection of HCWT.

Table 6-1 The mean, standard deviation and range of R630/R580 at 0, 24, 48 and 72 hours of simulated retail display and for the covariates tested.

	Number	Mean	St. Dev	Range
R630/R580 : 0 hr (30-60 min post-bloom)	4537	5.38	1.07	2.44 – 15.9
24 hr	4538	4.22	1.06	2.00 – 11.6
48 hr	4538	3.52	0.85	1.87 – 8.24
72 hr	4404	3.04	0.68	1.81 - 8.05
Covariates (units):				
pH_{24}	4393	5.66	0.15	5.23 – 6.67
Myoglobin (mg/g muscle)	2623	6.43	1.65	2.15 - 15.6
Iron (mg/100g muscle)	2625	20.0	3.39	8.12 - 45.1
Isocitrate dehydrogenase activity (µmol/min/g)	1804	5.03	1.56	1.43 - 11.3
Zinc (mg/100g muscle)	2625	24.1	4.50	12.0 - 44.9
Intramuscular fat (%)	2628	4.10	1.01	1.59 - 9.59
Hot carcass weight (kg)	4355	22.7	3.37	12.5 - 36.0
Shortloin muscle weight (g)	4400	360	95.6	157 - 1110
Shortloin fat weight (g)	4397	171	76.0	10.0 - 590
Age (days)	4403	248	76.0	134 - 503

6.3 Results

6.3.1 Correlations between R630/R580 measures over retail display

Lamb loin R630/R580 at 0 hours was weakly correlated with subsequent measures of R630/R580. R630/R580 at 0 hours had low simple correlations with R630/R580 at 24,

48 and 72 hours (Table 6-2). In contrast, R630/R580 measured at 24 hours was highly correlated with R630/R580 at 48 and 72 hours (Table 6-2). Partial correlation coefficients, that accounted for the fixed effects listed in Table 6-3, between R630/R580 at 0 hours and subsequent measures increased up to only 0.41 (Table 6-2). Accounting for the same fixed effects did not effectively alter the already high correlations between R630/R580 at 24, 48 and 72 hours (Table 6-2).

Table 6-2 Simple and partial correlation coefficients between R630/R580 on simulated retail display. Partial correlation coefficients account for site, year, slaughter group within site and year, sex and dam breed within sire type. Upper values represent simple correlations (above the diagonal), lower values represent partial correlations (below the diagonal).

R630/R580	0 hr	24 hr	48 hr	72 hr
0 hr	-	0.20	0.18	0.10
24 hr	0.40	-	0.82	0.81
48 hr	0.41	0.83	-	0.85
72 hr	0.32	0.80	0.87	-

Incorporating each carcass covariate listed in Table 6-1 into the multivariate models did not substantially alter the partial correlation coefficients between R630/R580 at 0, 24, 48 and 72 hours. The highest correlation coefficient of 0.40 was reached between R630/R580 at 0 and 72 hours with incorporation of muscle iron concentration. Incorporating lamb age, IMF and pH_{24} simultaneously produced a correlation coefficient of only 0.36 between R630/R580 at 0 and 72 hours. The correlation between R630/R580 at 0 and 24 hours was again highest when loin iron concentration was

accounted for (0.48), and reached 0.46 with incorporation of lamb age, IMF and pH_{24}. The high correlation coefficients between R630/R580 at 24, 48 and 72 hours did not increase by more than 0.04 when lamb age, IMF, pH_{24}, or any other carcass covariate was accounted for.

6.3.2 The effect of animal production factors on R630/R580 measures over retail display

The models for analysing production effects on lamb loin R630/R80 measures at 0, 24, 48 and 72 hours of display are outlined in Table 6-3. These models describe 62%, 55%, 58% and 58% of the variance in R630/R580 at 0, 24, 48 and 72 hours of display respectively.

Table 6-3 The base linear mixed effects models for lamb *m. longissimus lumborum* R630/R580 at 0, 24, 48 and 72 hours of simulated retail display.

Effect	NDF, DDF	F-values			
		0 hours	24 hours	48 hours	72 hours
Site	4, 776	23.7	88.4	153.7	86.1
Year	4, 3106	94.1	39.5	6.5	16.7
Slaughter group (site x year)	62, 776	58.2	49.1	40.5	43.1
Sex Dam breed (Sire type)	5, 776	13.9	35.5	35.8	33.4

NDF, DDF: numerator and denominator degrees of freedom; all effects have a *P*-value < *0.001*

6.3.3 Carcass trait effects on R630/R580 measures over retail display

The models described in Table 6-3 were used to test the association between carcass covariates and each time-point measure of R630/R580 (Figure 6-1). The magnitude of

change in R630/R580 measured across the range of a covariate was largest for pH_{24} of all the covariates measured at 24, 48 and 72 hours display but not at 0 hours (Figure 6-1). R630/R580 decreased as pH_{24} increased from 5.2 to 6 (Figure 6-1, $P < 0.05$). While pH_{24} had a substantial effect on R630/R580 at 0 hours, its impact on R630/R580 more than doubled from 0 to 24 hours display (Figure 6-1). Myoglobin concentration was positively associated with all R630/R580 measures across display (Figure 6-1, $P < 0.05$), though the magnitude of its impact reduced substantially with time across display. Increasing muscle myoglobin concentration from 4 to 12 g/100g muscle had the largest magnitude impact on R630/R580 at 0 hours (1.37 units) of all covariates measured, and the smallest magnitude impact on R630/R580 at 72 hours (Figure 6-1, $P < 0.05$).

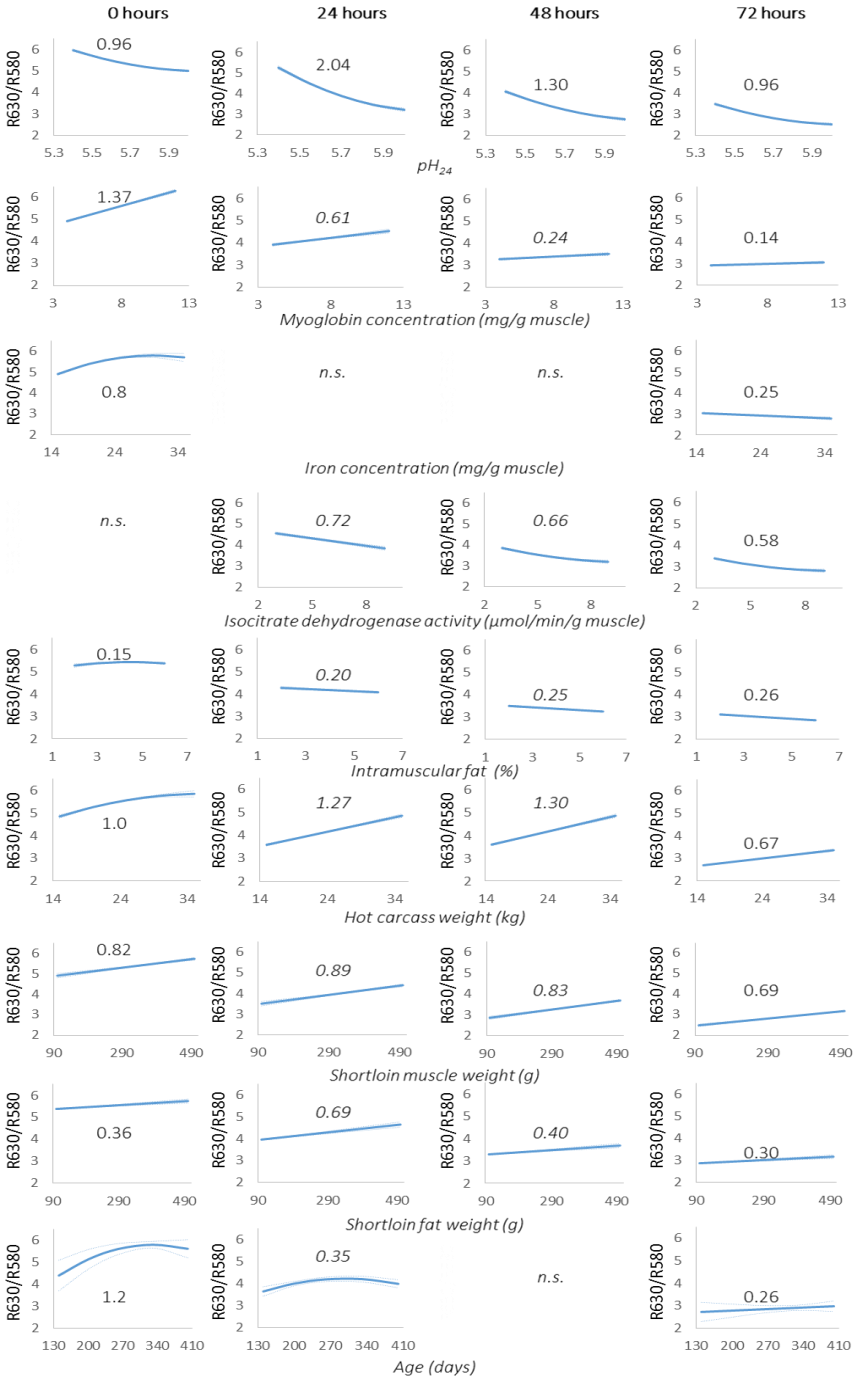

Figure 6-1 The associations between R630/R580 at 0, 24, 48 and 72 hours and lamb carcass covariates. Shortloin muscle and fat weight associations with R630/R580 measures were corrected for HCWT. Solid lines represent predicted means, dotted lines represent the standard error of the mean. The number above each figure represents the unit change in R630/R580 across the covariate range. All associations represented in this figure are statistically significant ($P < 0.05$). *n.s* represents a non-significant association ($P > 0.05$).

Lamb loin iron concentration was associated with R630/R580 only at 0 and 72 hours of display (Figure 6-1, $P < 0.05$). The effect of increasing iron concentration from 15 to 35 mg/100g of muscle) differed substantially between these two time points (Figure 6-1, $P < 0.05$). Increasing ICDH activity reduced R630/R580 from 24 to 72 hours of simulated retail display (Figure 6-1, $P < 0.05$), though was not associated with R630/R580 at 0 hours ($P > 0.05$). Increasing ICDH activity from 3 to 9 µmol/min/g of muscle had the greatest impact on R630/R580 at 24 hours display, though this effect diminished slightly with time across display. Loin zinc concentration was not associated with R630/R580 at any point during the simulated display ($P > 0.05$).

IMF concentration was negatively associated with all R630/R580 measures (Figure 6-1, $P < 0.05$). Increasing IMF from 2 to 6% had a relatively small impact on R630/R580, though its impact increased with time across display (Figure 6-1). Increasing HCWT from 15 to 35 kg increased R630/R580 across display (Figure 6-1, $P < 0.05$), though had a reduced magnitude of impact at 72 hours display. Increasing shortloin muscle weight from 100 to 500g relative to carcass weight increased R630/R580 across the display (Figure 6-1, $P < 0.05$). Increasing relative shortloin fat weight from 100 to 500g also increased R630/R580 across display ($P < 0.05$); though had only around half the magnitude of impact of increasing shortloin muscle weight over the same range (Figure 6-1). Increasing lamb age from 140 to 400 days increased R630/R580 at 0, 24 and 72 hours display (Figure 6-1, $P < 0.05$), though had a far greater magnitude of impact on R630/R580 at 0 hours than on subsequent measures.

6.3.4 Associations between R630/R580 measures over retail display and the influence of carcass traits on these associations

The associations between R630/R580 at 0 and 72 hours (a), R630/R580 at 0 and 24 hours (b), R630/R580 at 24 and 72 hours (c), and R630/R580 at 48 and 72 hours (d) are shown in Figure 6-2 ($P < 0.05$). The coefficients of determination (R-Square) are shown above each association, ranging from 0.10 between R630/R580 at 0 and 72 hours to 0.74 between R630/R580 at 48 and 72 hours of display (Figure 6-2). The R-Square and root mean square error of these associations did not change substantially when carcass traits were accounted for in the models (Table 6-4). The R-square for R630/R580 at 0 and 72 hours display increased from 0.10 up to 0.18 with incorporation of myoglobin, iron or IMF concentration and up to 0.20 with incorporation of shortloin fat weight (with HCWT) (Table 6-4). While incorporation of ICDH activity appeared to increase the R-square for R630/R580 at 0 and 72 hours the most to 0.22, ICDH activity was only measured in a smaller subset of lambs (n = 2881). In this subset of lambs, the R-square of the association between R630/R580 at 0 and 72 hours with no covariates accounted for was 0.20 rather than 0.10 (Table 6-4).

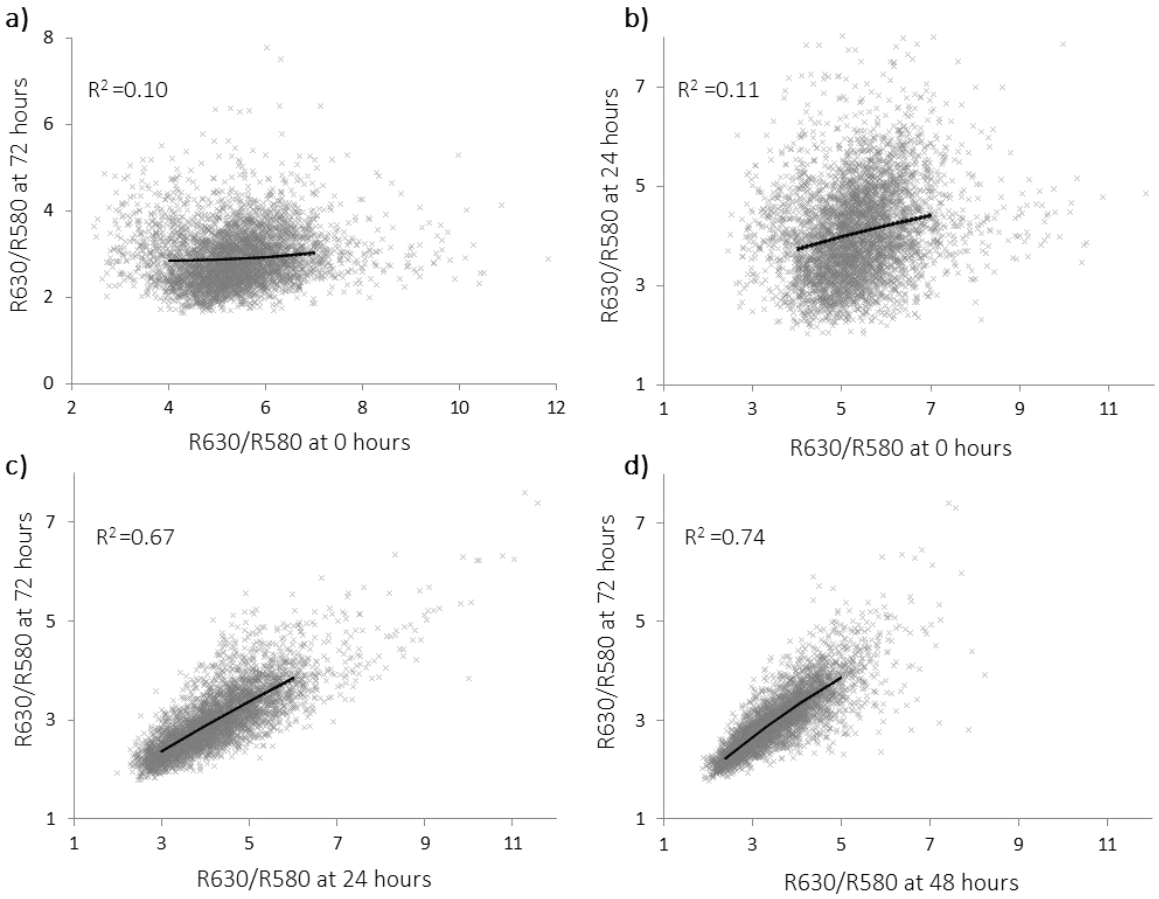

Figure 6-2 Associations and R-square between lamb loin R630/R580 measured at a) 0 and 72 hours of display; b) 0 and 24 hours display; c) 24 and 72 hours display and d) between R630/580 at 48 and 72 hours display.
Solid lines represent predicted means, dashed lines represent the standard error from the predicted mean (standard error too small to distinguish dotted lines clearly) and individual points represent the residuals values of the lambs (difference of raw value from the predicted means). The coefficient of determination (R-Square) for each association is shown above each graph.

Table 6-4 The influence of carcass traits on the coefficient of determination (R-square) and root mean square error (RMSE) of the associations between R630/R580 at different time points on simulated retail display.

	R630/R580 at 0 and 72 hours		R630/R580 at 0 and 24 hours		R630/R580 at 24 and 72 hours		R630/R580 at 48 and 72 hours	
	R-Square	RMSE	R-Square	RMSE	R-Square	RMSE	R-Square	*RMSE*
R630/R580 association:	0.10	0.64	0.11	1.00	0.67	0.39	0.74	0.35
Carcass trait incorporated:								
pH_{24}	0.17	0.62	0.18	0.96	0.67	0.39	0.74	0.35
Myoglobin (mg/g muscle)	0.18	0.64	0.15	1.01	0.71	0.38	0.78	0.33
Iron (mg/100g muscle)	0.18	0.64	0.14	1.02	0.71	0.38	0.77	0.34
Isocitrate dehydrogenase activity (µmol/min/g)^	0.22 (0.20)	0.66 (0.66)	0.16 (0.15)	1.02 (1.03)	0.72 (0.71)	0.39 (0.40)	0.78 (0.77)	0.35 (0.36)
Zinc (mg/100g muscle)	0.17	0.65	0.14	1.02	0.67˜	0.39˜	0.77	0.34
Intramuscular fat (%)	0.18	0.64	0.15	1.01	0.71	0.38	0.77	0.34
Hot carcass weight (kg)	0.13	0.63	0.16	0.97	0.68	0.38	0.74	0.35
Shortloin muscle weight (g)*	0.14	0.63	0.18	0.96	0.68	0.38	0.74	0.35
Shortloin fat weight (g)*	0.20	0.61	0.22	0.94	0.68	0.38	0.74	0.35
Age (days)	0.13	0.63	0.13	0.99	0.69	0.38	0.78	0.32

˜Zinc did not impact the association between R630/R580 at 24 and 72 hours (P>0.05). All other values represent a statistically significant ($P < 0.05$) effect of the carcass trait on the R630/R580 association

^Isocitrate dehydrogenase activity was measured in only 2881 lambs in this study. R-squared values and RSME of the association between R630/R580 measures within this subset of lambs are shown in brackets.

*Shortloin muscle and fat weights were analysed with hot carcass weight accounted for in the model.

6.4. Discussion

Contrary to our hypothesis, bloomed meat redness (R630/R580) at the start of display combined with measures of lamb age, meat pH24 and IMF concentration could not provide an accurate prediction of lamb meat browning after 3 days simulated retail display. This was shown in a number of ways. Firstly through the poor correlations between lamb loin R630/R580 measured at the start of display with all subsequent R630/R580 measures across the 3 day simulated display (Table 6-2). When these correlations were adjusted for fixed production effects (site and year of lamb production, slaughter group within site and year, sex and dam breed within sire type), or for the phenotypic carcass covariates (lamb age, pH_{24}, IMF, iron and myoglobin concentrations, ICDH activity, HCWT, relative shortloin muscle or fat weight) they were stronger, but not to the level that would suggest that they would be robust predictors of subsequent R630/R580 at 24, 48 or 72 hours display (Table 6-2). Secondly, through the general linear model associations between R630/R580 at time 0 versus the later time points (Figure 6-2) which described just 10 to 11% of the variation in colour and weren't considerably improved with the inclusion of fixed production effects or phenotypic carcass covariates in the model (Table 6-3). This suggests that even with considerable knowledge of animal background and carcass phenotype measures, there is little capacity to measure bloomed meat colour at the start of retail display to then predict the colour at 3, 2, or even 1 day into retail display. This might suggest that blooming takes longer than 30 minutes to complete or that oxidative

metabolism is less important in lamb meat than beef in relation to the variation in colour stability.

In contrast to this there were strong correlations between R630/R580 at 24, 48 and 72 hours display that varied little when corrected for fixed production factors or carcass phenotype measurements. Furthermore R630/R580 at both 24 and 48 hours were strongly associated with R630/R580 at 72 hours (Figure 6-2). In these models where only lamb sex and breed type were accounted for, R630/R580 measures at 24 and 48 hours described 67% and 74% of the variation in colour at 72 hours display. R630/R580 at 24 and 48 hours described up to 72 and 78% of 72 hour colour with the inclusion of carcass phenotype measurements (Table 6-4). These results suggest that even with no prior knowledge of animal information, colour measurements taken at 24 hours of retail display represent colour change due to metmyoglobin formation and thus would be useful to predict meat colour at 48 hours display and beyond.

The ability to predict retail meat browning using colour measured at 24 hours display rather than after blooming suggests that key factors determining meat browning are not manifesting their effects until some point between meat blooming and 24 hours post-oxygenation. Generally the greatest difference in individual covariate effects on R630/R580 at 0, 24, 48 and 72 hours were observed between 0 and 24 hours of display (Figure 6-1). Lamb age and pH_{24} for example had substantially different associations and impacts on R630/R580 at 0 hours compared to measures taken 24 hours later. The magnitude impact of increasing pH_{24} on R630/R580 more than doubled from 0 to 24 hours display. This suggests that the full effect of pH_{24} on meat redness was not apparent until 24 hours into retail display. The different effects of carcass traits on

R630/R580 at 0 and 24 hours likely relates to these time points representing different meat colour traits- the initial development of meat redness with blooming and the subsequent deterioration in meat redness with browning on retail display. Meat pH_{24} is an important determinant of both these colour traits, though exerts its influence on initial meat colour and on retail browning via different though related biochemical mechanisms. Increasing pH reduces meat lightness and redness due to changes to the physical structure of meat obstructing oxygen diffusion and due to increased oxygen consumption reducing oxygen availability to bind with myoglobin. Whereas the negative influence of pH on lamb meat colour stability mainly relates to free radical oxidative species that accumulate with *post-mortem* metabolism and trigger metmyoglobin formation. The reported effects of pH on meat colour remain conflicting, and the effect of pH on colour stability may differ between beef and lamb meat given that numerous studies demonstrating that increasing pH improves the colour stability of beef (Brown and Mebine 1969, Hunt, Sørheim et al. 1999, O'Grady, Monahan et al. 2001, Gutzke and Trout 2002, AMSA 2012) while studies demonstrate that increasing pH reduces the colour stability of lamb meat (Calnan, Jacob et al. 2014, Jacob, D'Antuono et al. 2014).

The effects of muscle myoglobin, iron and ICDH activity on R630/R580 across display may also relate to the different mechanisms underpinning bloomed meat colour development and subsequent retail browning. The substantial effect of myoglobin concentration on R630/R580 at 0 hours suggests the amount of pigment is the most important determinant of bloomed meat colour due to less variation in the type of myoglobin present at this time. Reflectance at 630 nm represents maximal reflection of

the red pigment oxymyoglobin whilst reflectance at 580 nm is the reflectance minimum for pure oxymyoglobin (AMSA 2012). The other myoglobin forms that determine surface meat colour, purple deoxymyoglobin and brown metmyoglobin are also reflected at these wavelengths to an extent, with moderate deoxymyoglobin reflection at 630 nm and metmyoglobin being the principle pigment reflected at 580 nm. The reduced impact of myoglobin on R630/R580 at 24 hours onwards suggests the relative concentrations of the different forms of myoglobin (deoxymyoglobin, oxymyoglobin and metmyoglobin) vary more at these times than at the start of display, so the amount of myoglobin pigment is less predictive for redness from 24 hours on retail display (O'Keeffe and Hood 1982, McKenna, Mies et al. 2005, Calnan, Jacob et al. 2014). Given the close association between myoglobin and iron concentration in muscle, this may in turn account for the moderate influence of iron on R630/R580 at the start of display, its lack of effect at 24 and 48 hours of display, and the small effect of iron at the end of display when the free-radical by-products of oxidative metabolism accumulate and trigger myoglobin oxidation. ICDH activity is more closely associated with oxidative metabolism than myoglobin pigmentation, which may explain its failure to influence initial R630/R580 measures and its moderate effect on subsequent colour measures across display. As with pH_{24}, the influence of myoglobin, iron and ICDH activity on bloomed meat colour development and subsequent meat browning are interrelated and difficult to fully distinguish.

Meat colour measured shortly after blooming and 24 hours later representing different meat colour traits may account for the weak correlations between R630/R580 at 0 hours and all subsequent measures of R630/R580, and the strong correlations between

R630/R580 at 24, 48 and 72 hours of display. Based on these results, measuring meat colour at 24 hours display could provide an excellent prediction of meat browning in the subsequent days of retail display. However, measuring meat colour after 24 hours of display is unrealistic in a commercial retail setting. Having only measured R630/R580 at 24 hour intervals in this study, the possibility remains that R630/R580 measured earlier in retail display could better predict subsequent retail meat browning. Measuring R630/R580 6 hours post-blooming for example, may prove more feasible in a retail setting. Until work has been undertaken to determine if R630/R580 measured between 0 and 24 hours could accurately predict meat browning, the value of R630/R580 may be limited to the use of 24 hour measures in driving genetic improvement of lamb meat colour stability via the development of a retail colour breeding value for lamb meat.

6.5 Conclusion

Lamb loin redness (R630/R580) measured at the start of retail display was poorly correlated with colour stability as indicated by R630/R580 at the end of the display period. Accounting for meat pH_{24}, IMF concentration and lamb age did not improve these correlations. Meat colour at the start of the display period was determined mainly by the amount of myoglobin pigment present, while meat colour after 72 hours display was influenced by factors such as pH and ICDH activity, presumably due to their effects on myoglobin oxidation. Therefore measuring bloomed meat colour is unlikely to be a useful tool for predicting colour stability in a commercial application. In contrast, meat colour measured at 24 hours display can accurately predict the extent of meat browning at 2 to 3 days of retail display. However, measuring meat colour after 24 hours on display is not

practical in a retail setting, thus these measures may only prove valuable in the development of a retail colour breeding value to reduce meat browning and the resultant economic losses suffered by the industry.

Acknowledgements

The authors gratefully acknowledge the financial support of the Cooperative Research Council for Sheep Industry Innovation. Considerable staff and resources were provided at each site of the Information Nucleus Flock by the NSW Department of Primary Industries, University of New England, Department of Primary Industries Victoria, SA Research & Development Institute and the Department of Agriculture and Food WA. The authors also wish to thank the people who assisted with site supervision, sample collection and measurement as well as abattoir staff. For the New South Wales sites; Dr. D. Hopkins, Dr. G. Refshauge, Mr. D. Stanley, Ms. S. Langfield, Mrs. T. Bird-Gardiner, Ms. T. Lamb, Mrs. E. Toohey and Mr. M. Kerr. For the Victorian sites; Dr. E. Ponnampalam, Mr.W. Brown, Mr. A Naththarampatha and Mr. M. Kerr. For the Western Australian sites; Dr. Kelly Pearce, Mr. K. Hart, Mr. M. Boyce and Mr. A. Williams.

Chapter 7. Dietary vitamin E supplementation improves the colour stability of lamb *longissimus* meat following up to 70 days of chilled storage

Abstract

Rapid browning limits the retail display of lamb meat following extended chilled storage that is typical of international shipment. Lamb stored for short periods browns more rapidly on retail display with high levels of marbling (intramuscular fat), oxidative capacity or pH and less rapidly with high vitamin E concentration. The ability of dietary Vitamin E supplementation to negate these negative impacts on browning is unknown, particularly in long stored lamb meat. Lambs (n=132) of mixed breed type were fed basal or high concentrations of vitamin E (30 or 275 mg/kg feed) for 56 days prior to slaughter. The *m.longissimus lumborum* was sampled for measurement of vitamin E, oxidative enzyme isocitrate dehydrogenase activity, intramuscular fat, pH and retail meat colour. Colour samples were vacuum packaged for storage at -1°C for 5, 35 and 70 d, before being re-sliced, wrapped and placed under stimulated retail display where meat redness (R630/R580) was measured 24 hourly for 72 hours. High muscle vitamin E reduced meat browning at 48 and 72 hours display regardless of storage time ($P < 0.05$), however had a slightly greater effect in meat stored for 35 d than 5 or 70 d. High intramuscular fat increased browning in 5 day stored lamb ($P < 0.05$) and vitamin E had a greater effect improving the display colour in this highly marbled meat. These results demonstrate that

dietary vitamin E supplementation will improve the display colour of highly marbled or long stored lamb meat.

7.1 Introduction

The red colour of lamb meat is crucial to consumer appeal. The rapid browning of lamb meat on retail display is an important problem for lamb meat industries as consumers associate brown meat with reduced quality and freshness (Faustman and Cassens 1990). Retailers therefore commonly limit the retail display of overwrapped lamb meat to only 2 days, at which point lamb of otherwise premium quality is discounted or downgraded to ensure its sale.

The appealing red colour of lamb meat forms with blooming, when a meat surface is exposed to oxygen causing the oxygenation of myoglobin into the red pigment oxymyoglobin. Following blooming, oxidative *post-mortem* metabolism depletes oxygen concentrations and generates oxidative free radical by-products in meat that trigger the oxidation of myoglobin into brown metmyoglobin. The rate of myoglobin oxidation or colour stability of lamb meat is influenced by a number of factors including meat storage or ageing prior to retail display (Suman and Joseph 2013).

Lamb meat may be stored in vacuum-packaging at low temperatures (-1.5 to 0°C) for up to 12 weeks prior to retail display (MLA 2005), facilitating the international shipment of chilled lamb meat. However, increasing meat storage reduces the already limited colour stability of lamb meat on retail display (Wulf, Morgan et al. 1995, Jose, Pethick et al. 2008, King, Shackelford et al. 2012) and thereby limits the marketability of long-stored lamb meat. The mechanism underpinning the reduction in colour stability with meat storage is unclear, through may relate to increased lipid oxidation with meat storage

(Eikelenboom, Hoving-Bolink et al. 2000, Ismail, Lee et al. 2008, Vitale, Pérez-Juan et al. 2014). Lipid oxidation generates reactive secondary products that accelerate myoglobin oxidation and meat browning (Faustman, Sun et al. 2010).

Muscle factors linked to lipid peroxidation such as intramuscular fat (IMF) concentration can also reduce the colour stability of lamb meat (Calnan, Jacob et al. 2014, Jacob, D'Antuono et al. 2014). Recent work identifying the negative impact of pH_{24} and muscle oxidative capacity on the colour stability of lamb loin meat (Calnan, Jacob et al. 2014) may also relate to their impact increasing metabolic activity (Tang, Faustman et al. 2005) and thereby production of reactive oxygen species in meat. The negative impact of increasing IMF, muscle oxidative capacity (measured via oxidative enzyme isocitrate dehydrogenase or ICDH activity) and pH_{24} on retail colour stability has only been demonstrated in short-stored lamb meat (Calnan, Jacob et al. 2014), though these factors are likely to also reduce the colour stability of lamb meat following extended storage.

Dietary vitamin E supplementation is a proven means of increasing the colour stability of lamb meat on display (Wulf, Morgan et al. 1995, Guidera, Kerry et al. 1997, Lauzurica, de la Fuente et al. 2005, Jose, Jacob et al. 2016), due to the capacity of vitamin E to neutralise oxidative free radicals that trigger myoglobin oxidation. Increasing muscle vitamin E minimises lipid oxidation during the display of lamb loin following 4 weeks of chilled storage (Ponnampalam, Butler et al. 2014), however the capacity of vitamin E to mitigate lipid and myoglobin oxidation in lamb meat stored for longer periods has not been demonstrated. This study aims the evaluate the ability of dietary Vitamin E supplementation to improve the colour stability of long-stored lamb meat with high IMF, ICDH activity and pH_{24}, given the likely increased oxidative load triggering browning in this meat. We hypothesised that vitamin E supplementation would improve the retail

colour stability of lamb loin to a greater extent following extended storage. Additionally we hypothesised that vitamin E supplementation would have a greater effect on the colour stability of lamb loin with high IMF, ICDH activity and pH_{24}.

7.2 Materials and methods

7.2.1 Animal, genetic and production details

Data were collected from 132 lambs in Meat and Livestock Australia's Research Flock in Katanning, Western Australia (AEC No. 2-13-08). Industry-proven sires (n = 66) of Terminal, Maternal and Merino breed types were used to artificially inseminate Merino and Border-Leicester Merino dams. Sires were selected based on their breeding values for muscling (post-weaning eye muscle depth) and fatness (post-weaning fat depth) to produce progeny divergent in muscle IMF concentration and ICDH activity given that sire breeding values for muscling and fatness are associated with IMF concentrations (Pannier, Pethick et al. 2012) and ICDH activity (Kelman, Pannier et al. 2014) in the loin muscle of lambs.

Two lambs were selected from each sire so that sires could be equally represented in each vitamin E supplementation group. All female lambs (n = 44) were of Terminal sire type as Maternal and Merino sired lambs were retained for breeding purposes, meaning that effective comparisons between sexes could only be made within Terminal sired lambs. The lambs were raised on extensive or broad acre pastures, with grain provided when pasture supplies were limited. Lambs were fed dry hay in a paddock devoid of green pasture (that contains abundant vitamin E) for 6 weeks to reduce their intramuscular vitamin E content according to the work of Fry *et al.* (1993) Lambs were gradually

acclimatised to the complete pelleted ration containing basal vitamin E content for 2 weeks prior to the commencement of supplementation.

7.2.2 Dietary vitamin E supplementation

Lambs were divided into 12 pens (6 pens per treatment, 11 lambs per pen), balanced for breed type, with similar sized animals penned together. The average live weight of a pen ranged from 44 kg to 55 kg, with an average 8 kg range in live weight within pens. The lambs were fed a complete pelleted ration only for 8 weeks prior to slaughter, identical apart from the vitamin E content. Lambs were fed pellets containing basal vitamin E concentrations of 30 mg/kg feed or were supplemented with 275 mg of vitamin E/kg feed. The control group of lambs were fed basal levels of Vitamin E to prevent deaths from white muscle disease according to the National Research Council's nutrient requirements of sheep (NRC 1985). The dose and duration of high vitamin E supplementation was based on the work of Jose *et al*. (2016). Feed intake of individual lambs or by pen was not measured.

7.2.3 Slaughter and muscle sampling

Lambs were fasted for 6 hours prior to transport to a commercial abattoir for slaughter. Lambs ranged from 273 - 287 days of age and had a mean carcass weight of 23 kg (± 0.3) at slaughter. Within 4 hours of slaughter a 1 g portion of the *m. longissimus thoracis et lumborum* was sampled at the position of the 12th rib and snap-frozen in liquid nitrogen for measurement of ICDH activity according to the method of Briand (1981). At 24 hours *post-mortem* the pH of the *m. longissimus thoracis et lumborum* was measured (pH_{24}) by inserting a TPS WP-80 probe into the centre of the muscle at the position of the 12th rib,

as described by Pearce et al. (2010). The pH electrode was calibrated with standardised buffers (pH 4.0 and 7.0) prior to measurement.

The *m. longissimus thoracis et lumborum* (loin muscle) was removed (caudal from the 12th rib region to the lumbar sacral junction) for measurement of vitamin E concentration, IMF content and retail colour. Vitamin E concentration was measured using high performance liquid chromatography with fluorescence detection, a modified method of McMurray et al. (1980). IMF was determined using 40 g of loin muscle sampled from the caudal (lumbar sacral) portion of the muscle. All subcutaneous fat and epimysium (silver-skin) were removed and the samples were stored at –20°C until freeze drying using a Cuddon FD 1015 (Cuddon Freeze Dry, NZ). IMF was determined using 40 g of loin muscle sampled from the caudal (lumbar sacral) portion of the muscle following removal of all subcutaneous fat and epimysium (silver-skin). The samples were stored at –20°C until freeze drying using a Cuddon FD 1015 (Cuddon Freeze Dry, NZ) and determination of IMF using a near infrared procedure in a Technicon Infralyser 450 (19 wavelengths), using the method described by Perry et al. (2001). IMF concentration is expressed as a percentage of fresh muscle weight.

7.2.4 Meat colour measurement

The remaining *m. longissimus lumborum* was excised for measurement of retail colour following storage. Three samples of loin muscle (at least 50 mm in length, 50 mm in width and 30 mm in depth) were collected from each lamb at 24 hours *post-mortem* for meat colour measurement. Each sample was individually vacuum packaged using an 11 L/min vacuum (Orved Eco Vacuum pro) in clear gas-impermeable plastic (20/80-100 microns, transparent polyamide air impenetrable exterior, polyethylene food approved

interior, water vapour transmission rate measured at 23°C and 85% R.H – 2,6 gr/mq – 24hr, oxygen permeability measured at 23°C and 0% R.H – 50 cm3/mq – 24hr – bar) for storage at -1°C for 5, 35 and 70 days, representing short, medium and long storage periods. The samples stored within a box in the dark within an export abattoir chiller, where there was limited flux in air movement or temperature.

At the end of each storage period samples were re-sliced perpendicular to muscle fibre orientation to create a freshly cut meat surface. The samples were placed on black Styrofoam trays (12 x 12 cm) with the fresh-cut surface facing upwards and wrapped with oxygen-permeable polyvinyl chloride film (Resinite "DHW" Meat AEP, 15 um, oxygen transmission rate of 35650 – 46500 cc/m^2/24 h). The loin samples were then placed under a simulated retail display for a period of 72 hours; where temperature and light conditions were designed to simulate those commonly encountered in Australian retail stores. The loin samples were displayed on a flat horizontal surface within a walk-in chiller (3.8 x 3 x 4 m) set to a temperature of 4°C with no defrost cycle. At this setting the temperature fluctuated between 2 and 6°C. The light source consisted of 8 Nelson Fluorescent Meat Display BRB Tubes, 58 W and 1520 mm in length, suspended 1.5 m above the loin samples. These tubes provided a light intensity of 1000 Lux as measured with an Electronics Light Metre Q1367, with a diffuser fitted to ensure even distribution of light and an actual colour temperature of 4000K.

Muscle sample were allowed to bloom for 30 - 60 minutes before meat colour was measured using a Hunterlab spectrophotometer (XE Plus, Cat. No. 6352, model No. 45/0-L). Meat redness (R630/R580) was calculated from reflectance readings (% reflectance at 630nm / % reflectance at 580nm) measured every 24 hours over the 72 hour display (AMSA 2012). Two spectrophotometric measures were taken of each sample at 90° on

the horizontal plane and R630/R580 values were averaged for statistical analysis. R630/R580 values greater than 1 represent meat redness due to oxymyoglobin and deoxymyoglobin while values approaching 1.0 represent predominantly metmyoglobin pigment and a brown meat colour. Measures were taken in the chiller with the 3.18 cm aperture flush against the overwrapped meat surface. The light source of the Hunterlab was set at "D65" with the observer set to 10°. The instrument was calibrated using black glass and white ceramic tiles according to manufacturer directions.

7.2.5 Statistical analysis

R630/R580 data were analysed using linear mixed effects models (SAS Version 9.1, SAS Institute, Cary, NC, USA). The base model included fixed effects for sire type and dam breed and sex (4) within sire type (3), covariate effects for pre-display storage time and time on simulated display, as well as relevant first order interactions between these terms and squared terms. Sire and dam identification were included as random terms in the model. Non-significant interactions ($P > 0.05$) were removed in a step-wise fashion to form the base model.

This base model was used to test for associations between R630/R580 and intramuscular vitamin E concentration, IMF concentration, ICDH activity and pH_{24}. Individual vitamin E concentrations were analysed as a covariate rather than as a fixed effect of supplementation group. The covariates were tested one at a time in the base model along with all relevant first order interactions with fixed effects. The effect of intramuscular vitamin E concentration on R630/R580 was then tested in combination with IMF, ICDH activity and pH_{24}. Vitamin E concentration had a significant interaction with intramuscular fat % ($P < 0.05$), not with ICDH activity and pH_{24} ($P > 0.05$). Figures 2

and 6 were produced by outputting the predicted mean of R630/R580 at the lower and upper end of the ranges in vitamin E (0.8 and 4.0 mg/g of muscle) and IMF concentration (2.5 and 5.5%) (Figure 7-1).

7.3. Results

The mean, standard deviation and range of intramuscular vitamin E concentration, IMF, pH_{24} and ICDH activity for all lambs and within each vitamin E supplementation group are shown in Table 1. Supplementing lambs with high vitamin E in their diet for 8 weeks prior to slaughter increased vitamin E concentration of the loin muscle ($P < 0.05$, Table 7-1), however loin IMF, pH_{24} and ICDH activity did not differ between lamb treatment groups ($P > 0.05$, Table 7-1).

Table 7-1 The mean, standard deviation and range in lamb loin vitamin E concentration, intramuscular fat %, pH_{24} and isocitrate dehydrogenase (ICDH) activity.

	All lambs (n=132)			Basal group (n=66)		Supplemented group (n=66)	
Muscle covariate	Mean	St.Dev.	Range	Mean	St.Dev.	Mean	St.Dev.
Vitamin E (mg/g muscle)	2.26	1.19	0.64 – 4.54	1.18^	0.49	3.34^	0.49
Intramuscular fat (%)	3.83	0.92	1.87 – 6.23	3.80	0.90	3.85	0.95
pH_{24}	5.61	0.09	5.49 – 6.11	5.60	0.08	5.61	0.09
ICDH activity (µmol/min/g)	3.57	0.70	1.43 – 5.9	3.60	0.70	3.54	0.71

^ values differ between lamb treatment groups ($P < 0.05$).

Intramuscular vitamin E concentrations were not correlated with IMF (Fig. 7-1), pH_{24} or ICDH activity in this data. Increasing meat storage from 5 days to 70 days prior to retail display reduced R630/R580 from 48 hours of retail display ($P < 0.05$, Table 7-2).

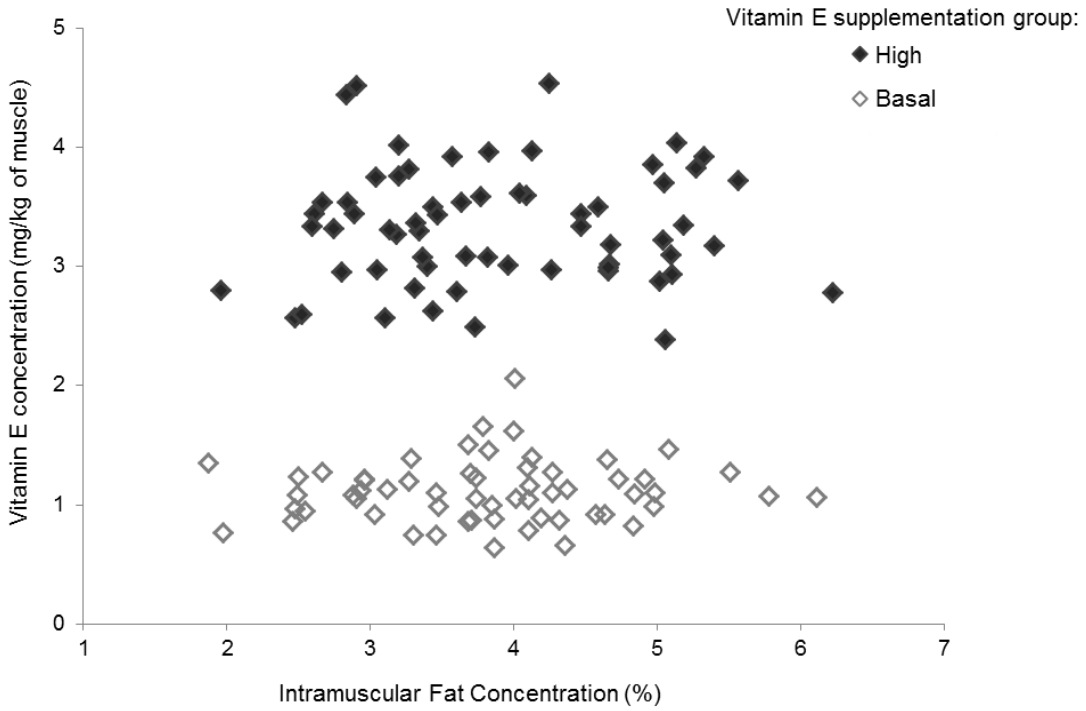

Figure 7-1 Loin intramuscular fat % relative to vitamin E concentration in lambs following dietary supplementation with high (275mg/kg feed) and basal (30mg/kg feed) concentrations of vitamin E.

Table 7-2 Lamb loin reflectance values (R580, R630 and R630/R580), with standard deviation in brackets, at each time point over the simulated retail display following 5, 35 and 70 days of chilled storage.

Display time (hours)	5 days storage			35 days storage			70 days storage		
	R580	R630	R630/R580	R580	R630	R630/R580	R580	R630	R630/R580
0	4.52 (1.44)	25.60 (3.38)	6.18[a] (1.89)	5.72 (0.88)	27.86 (2.96)	4.94[b] (0.64)	5.65 (1.1)	28.16 (2.78)	5.11[c] (0.79)
24	6.24 (0.95)	25.23 (3.13)	4.12[d] (0.72)	6.25 (0.99)	24.76 (2.64)	4.04 [d] (0.68)	5.51 (1.19)	20.96 (2.91)	3.97 [d] (0.96)
48	5.53 (1.05)	21.73 (3.41)	4.03[e] (0.79)	6.63 (1.06)	20.94 (2.76)	3.23[f] (0.63)	7.82 (1.49)	19.02 (2.57)	2.52 [g] (0.56)
72	6.31 (1.15)	20.19 (3.10)	3.28[h] (0.64)	7.49 (1.45)	18.67 (2.67)	2.59[i] (0.65)	8.02 (1.83)	17.15 (2.49)	2.25[j] (0.6)

Different letter superscripts in the same row or column represent where R630/R580 differed between storage periods or between time points on retail display ($P < 0.05$).

7.3.1 The influence of vitamin E on the display colour of lamb loin following 5, 35 and 70 days storage

Intramuscular vitamin E had a significant impact on loin meat R630/R580 at 48 and 72 hours of display independent of storage time ($P < 0.05$, Figure 7-2). Increasing muscle vitamin E concentration from 0.8 to 4.0 mg/g increased R630/R580 at 72 hours display by 0.58, 0.60 and 0.50 units in meat stored for 5, 35 and 70 days respectively ($P < 0.05$, Fig. 7-2).

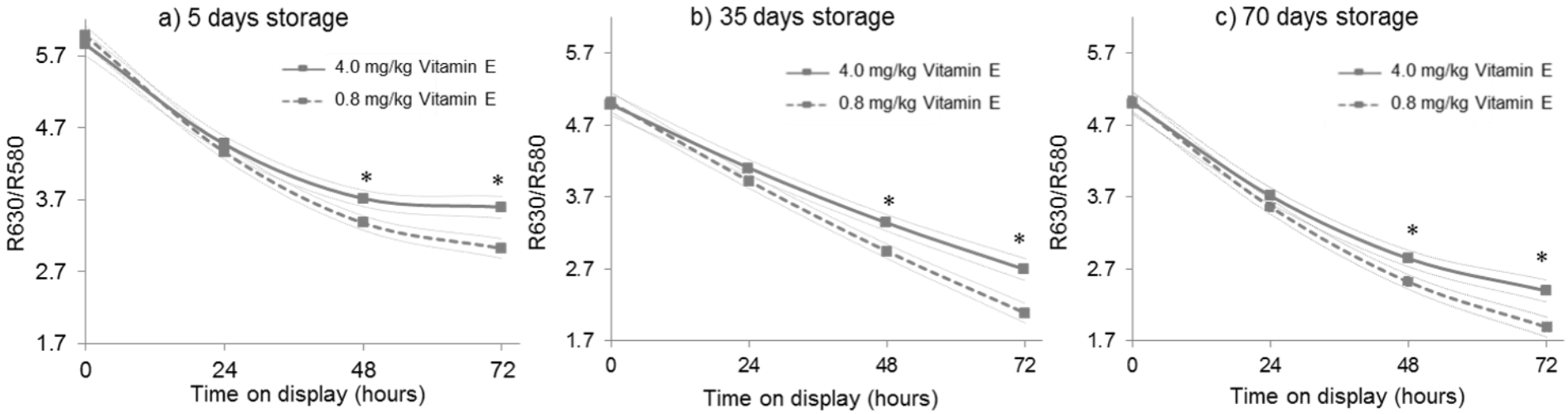

Figure 7-2 The effect of increasing vitamin E concentration from 0.8 to 4.0 mg/g of muscle on the R630/R580 of lamb loin over a 72 hour display following a) 5 days, b) 35 days, and c) 70 days of chilled storage of the loin prior to further processing for retail display.

Lines represent predicted means at 0.8 and 4.0mg of vitamin E per g of muscle ± SE. * represents where vitamin E concentration had a significant impact on R630/R580 ($P < 0.05$).

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7.3.2 The influence of IMF, ICDH activity and pH_{24} on the display colour of lamb loin following 5, 35 and 70 days chilled storage

Increasing lamb loin IMF % reduced R630/R580 on display following 5 days of chilled storage ($P < 0.05$). Increasing IMF from 2 to 6% in this short-stored meat reduced R630/R580 by 0.53 units at 72 hours of display ($P < 0.05$, Fig. 7-3). However, IMF % did not influence the R630/R580 of lamb loin on display following 35 or 70 days storage ($P > 0.05$).

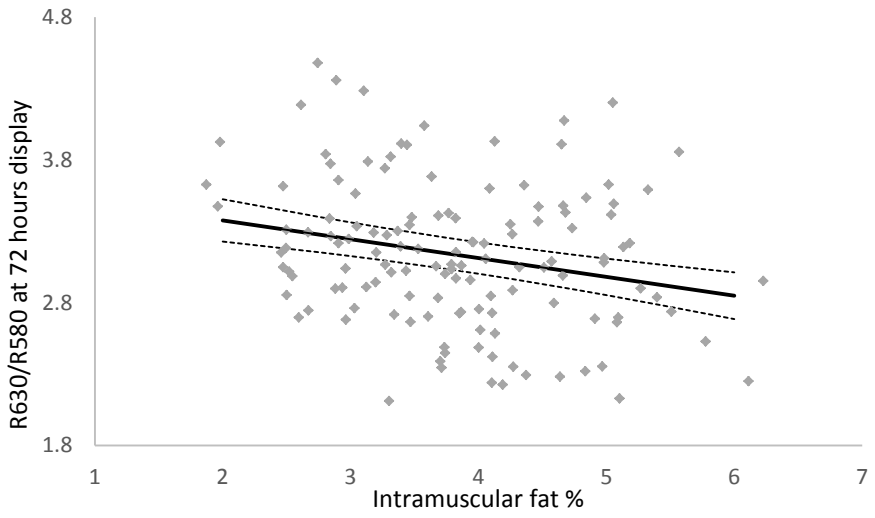

Figure 7-3 The effect of intramuscular fat on R630/R580 at 72 hours display in 5 day stored lamb loin (P<0.05). Lines represent predicted means ±SE. Points represent residuals from the predicted means.

Increasing ICDH activity reduced R630/R580 on display in lamb loin following 35 days of chilled storage, where increasing ICDH activity from 2 to 5 µmol/min/g of muscle reduced R630/R580 by 1.1 units at 72 hours display ($P < 0.05$, Fig. 7-4). Loin ICDH activity did not however impact the R630/R580 of meat following 5 or 70 days storage ($P > 0.05$). Similarly, loin pH_{24} influenced display R630/R580 in meat stored for 35 days ($P < 0.05$, Fig. 5), not in meat stored for 5 or 70 days ($P > 0.05$). In 35 day stored

meat, increasing pH_{24} from 5.5 to 5.8 increased R630/R580 at 72 hours display by 1.0 unit ($P < 0.05$, Fig. 7-5).

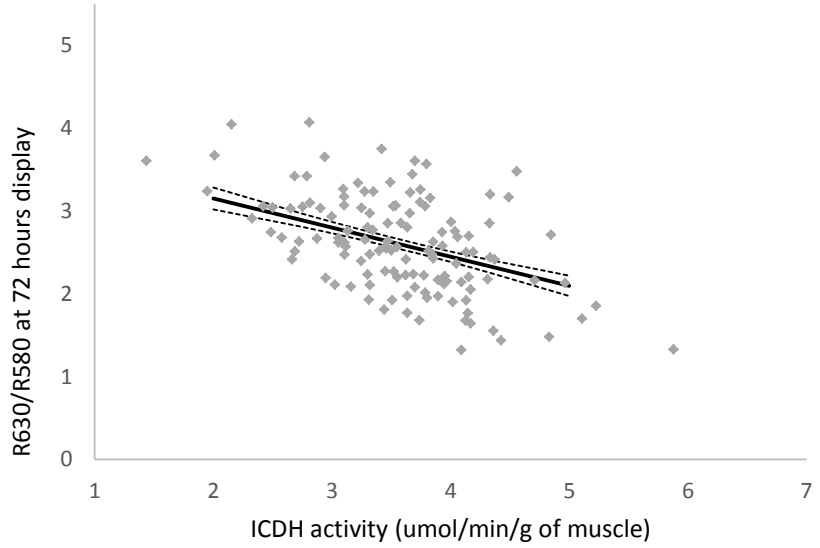

Figure 7-4 The effect of isocitrate dehydrogenase activity on R630/R580 at 72 hours display in 35 day stored lamb loin ($P < 0.05$).
Lines represent predicted means ±SE. Icons represent each residual from the predicted means.

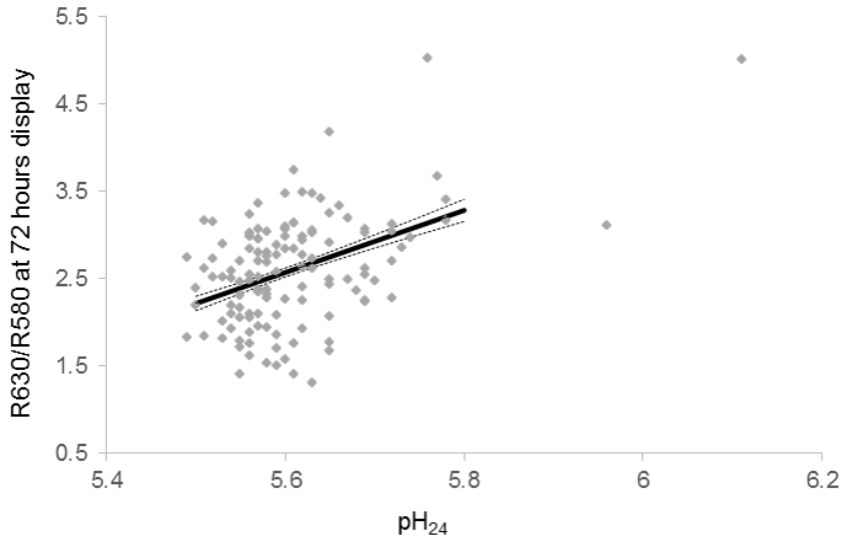

Figure 7-5 The effect of pH_{24} on R630/R580 at 72 hours display in 35 day stored lamb loin ($P < 0.05$). Lines represent predicted means ±SE. Icons represent each residual from the predicted means.

7.3.3 The influence of vitamin E on the display colour of high intramuscular fat, ICDH activity and pH_{24} loin following 5, 35 and 70 days chilled storage

Despite the influence of ICDH activity and pH_{24} on R630/R580 in lamb loin displayed following 35 days storage ($P < 0.05$, Fig. 7-4 and 7-5), the effect of vitamin E on R630/R580 was not influenced by ICDH activity or pH_{24} in this meat ($P > 0.05$). The influence of vitamin E on R630/R580 of lamb loin stored for 5 days was influenced by IMF concentration ($P < 0.05$, Fig. 7-6). Muscle vitamin E influenced the R630/R580 of high IMF (5.5%) meat at all time points on display, increasing R630/R580 by 0.87 units at 0 hours and by 1.47 units at 72 hours display ($P < 0.05$, Fig. 7-6). In low IMF (2.5%) meat, increasing muscle vitamin E only influenced R630/R580 at 48 and 72 hours display ($P < 0.05$, Fig. 7-6).

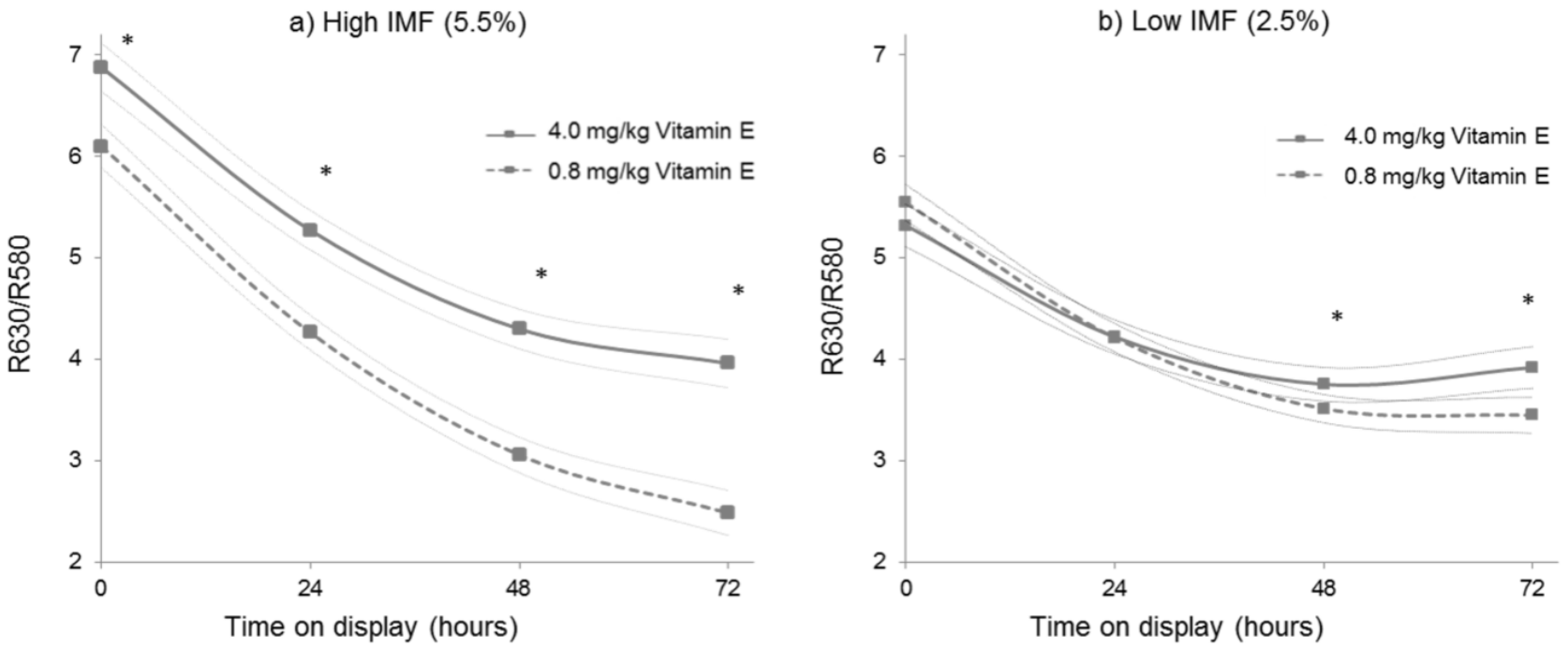

Figure 7-6 The effect of increasing muscle vitamin E concentration from 0.8 to 4.0 mg/g of muscle on the R630/R580 over 72 hours display in a) high intramuscular fat (5.5% IMF) and b) low intramuscular fat (2.5% IMF) meat following 5 days of chilled storage.

Lines represent predicted means at each vitamin E concentration ±SE. * represent a statistically significant difference in R630/R580 at different vitamin E concentrations ($P < 0.05$).

7.4 Discussion

7.4.1 The influence of vitamin E on the display colour of lamb loin following 5, 35 and 70 days chilled storage

Increasing intramuscular vitamin E via dietary supplementation successfully improved the colour stability of lamb loins following 5, 35 and 70 days of chilled storage. However, contrary to our hypothesis, vitamin E supplementation did not have a greater effect on the colour stability of lamb loin with increasing storage time prior to retail display. Instead, increasing vitamin E concentration from 0.8 to 4mg/g of muscle had a similar impact on meat redness (R630/R580) on display regardless of storage time. Increasing vitamin E had the largest magnitude effect on loin redness following 35 days storage, though this was only marginally higher (18%) than its lowest effect following 70 days storage. Increasing muscle vitamin E was hypothesised to have an enhanced impact on retail colour stability with increased storage time based on an anticipated increased oxidative load in this meat (Wulf, Morgan et al. 1995) providing greater opportunity for the antioxidant activity of vitamin E to prevent myoglobin oxidation and meat browning. In part this aligns with our results, given the enhanced effect of vitamin E in lamb loin stored for 35 days relative to 5 days, however does not account for the reduced effect of vitamin E on displayed meat colour after 70 days storage.

The failure of vitamin E supplementation to have an enhanced impact on the colour stability of long stored meat could relate to a high oxidative load overwhelming the antioxidant capacity of the vitamin E (Wulf, Morgan et al. 1995), to an incorrect assumption that increased oxidation underpins the reduced colour stability of long-

stored meat, and/or to a reduced concentration or efficacy of vitamin E in meat following extended storage. Oxidation was not measured in this study, while vitamin E concentration was measured in lamb loins at 24 hours *post-mortem* only, thus the concentration or antioxidant potential of vitamin E after extended meat storage is unknown. A recent study of Jacob *et al.* (2015, unpublished) measured vitamin E concentration in lamb loins at 24 hours *post-mortem* and after 60 days storage under similar conditions (vacuum packaged at -1°C) and found no change in vitamin E concentration following storage. Thus it seems unlikely that a loss in vitamin E potency over 70 days storage would account for the less than expected efficacy of high vitamin E on display colour in this meat.

Despite the slightly reduced effect observed after extended storage, vitamin E supplementation did successfully delay browning and thus extend the colour shelf life of all lamb loins in this study. Supplementing lambs with vitamin E in this study provided around 5 hours additional shelf life in long-stored lamb loin, 12 hours in medium-stored loin and 18 hours in short-stored loin, based on work demonstrating consumer acceptance of displayed lamb meat at R630/R580 of 3.3 or above (Khliji, Van de Ven et al. 2010). Short-stored lamb loin with high vitamin E concentration maintained an acceptable colour across the 72 hour display, thus a longer display is needed to appreciate the full extent of the benefits of vitamin E supplementation on the display colour of short-stored lamb meat. The results of this study suggest that vitamin E supplementation may be more useful for lamb product marketed domestically than for product marketed overseas after extended shipping. Vitamin E supplementation could extend the colour shelf life of short-stored lamb loin from the current 2 day limit to over 3 days of retail display, particularly where animals are not fed green feed prior to slaughter and thus have low muscle vitamin E levels.

Given that 70 days is considered close to the upper limit for chilled lamb storage prior to retail display, and that vitamin E had maximal impact on the colour stability of meat stored for 35 days, it is likely that vitamin E supplementation can substantially improve the retail colour of all fresh stored lamb products. Higher supplementation rates of vitamin E might be indicated for long-stored lamb meat. The improvement in display colour of meat with vitamin E supplementation in this study are likely conservative estimates given that lower intramuscular vitamin E levels were produced in our high vitamin E supplementation group than has been reported in other vitamin E supplementation studies. Lambs receiving high vitamin E supplementation had a mean vitamin E concentration of 3.34 mg/kg of loin muscle; below the threshold cited to minimise lipid oxidation (3.45 mg/kg) (Ponnampalam *et al*, 2014) and to positively influence meat colour (3.5-4mg/kg) (Arnold, Scheller et al. 1992, Wulf, Morgan et al. 1995). Additionally, the mean vitamin E concentration achieved with supplementation in this experiment were substantially lower than concentrations described in other vitamin E supplementation experiments, such as > 5 mg/kg muscle produced by Jose *et al*. (2016) using similar vitamin E supplementation rates as in this study.

The lower than expected intramuscular vitamin E concentrations in lambs fed high vitamin E diets may relate to the availability of vitamin E in the pelleted ration or to individual feed intake. Though individual feed intake was not measured, lamb weights were monitored to ensure the lambs were gaining weight and approaching slaughter weight. The impact of muscle vitamin E on the display colour of meat following storage has been shown when vitamin E concentrations increase from 0.8 to 4 mg/kg of muscle, rather than as an effect of supplementation group given the relatively low mean vitamin E content of supplemented lamb meat. The influence of dietary vitamin E supplementation may still have been underestimated in this study given that dietary

vitamin E supplementation can produce intramuscular vitamin E concentrations > 5mg/kg of muscle (Jose *et al.* 2016).

7.4.2 The influence of IMF, ICDH activity and pH_{24} on the display colour of lamb loin following 5, 35 and 70 days chilled storage

Contrary to our hypothesis, increasing IMF, pH_{24} and muscle oxidative capacity or ICDH activity was not consistently associated with reduced colour stability of lamb loin on retail display in this study. In lamb loin stored for 5 days prior to display, only IMF % was associated with colour stability on retail display, not pH_{24} or ICDH activity. Increasing IMF reduced meat redness across retail display in this study, aligning with previous publications (Calnan, Jacob et al. 2014) and the notion that IMF is associated with lipid and myoglobin oxidation. The reason that pH_{24} did not influence the colour stability of short-stored lamb loin as in prior studies may relate to the limited high pH_{24} meat (> 5.8) produced in this study.

In contrast, the display colour of lamb loin following 35 days of chilled storage was influenced by ICDH activity and pH_{24} but not by IMF. Increasing ICDH activity was associated with reduced colour stability in this medium-stored loin meat. The reason that this marker of oxidative capacity only influenced retail colour following medium storage is unclear, particularly given that far larger studies have established the negative association between ICDH activity and R630/R580 in lamb loin displayed following 5 days of chilled storage (Calnan, Jacob et al. 2014). Additionally, Ponnampalam *et al.* (2014) reported that heme iron content (a marker of muscle oxidative capacity) increased lipid oxidation when muscle vitamin E concentrations were < 2.95mg/kg muscle. The lack of association between ICDH activity and thus oxidative capacity and

display colour in short-stored loin in this study does support that the effect of IMF on display colour is related to changes in lipid oxidation rather than to any associated change in muscle oxidative capacity.

The influence of pH_{24} on retail colour of lamb loin following 35 days of chilled storage was entirely unexpected. Increasing pH_{24} improved the display colour of medium-stored lamb loin. This finding conflicts with previous work demonstrating that increasing pH_{24} substantially reduces colour stability of lamb loin displayed after 5 days of storage (Calnan, Jacob et al. 2014, Jacob, D'Antuono et al. 2014). An increase in meat pH with meat storage has been reported (Callejas-Cárdenas, Caro et al. 2014), thus it is possible that pH measured at 24 hours *post-mortem* is an inaccurate measure of lamb meat pH after extended storage.

The retail colour of lamb loin following 70 days of chilled storage was not influenced by IMF, ICDH activity or pH_{24}, contrary to our expectations. The reason that these factors do not influence the colour stability of lamb meat following such extended storage is unclear, though could suggest no relationship between these factors and oxidation reactions in long-stored meat or that the increased free radical production associated with these factors was insignificant relative to the overall increased oxidative load in long-stored meat. The lack of effect of IMF in long-stored meat is promising as it suggests that current selection to improve the taste quality of lamb meat by increasing IMF levels to 4-6% will not worsen the retail colour stability of the meat following extended storage.

7.4.3 The influence of vitamin E on the display colour of high intramuscular fat, ICDH activity and pH_{24} loin following 5, 35 and 70 days chilled storage

Though increased ICDH activity was associated with reduced retail colour stability in medium-stored lamb meat, the positive effect of vitamin E on meat colour was not enhanced in this meat. The reason that vitamin E did not negate the negative influence of muscle oxidative capacity on colour stability is unclear. The impact of vitamin E on retail colour in medium-stored lamb loin was not influenced by pH_{24} either, despite the impact of pH on retail colour in this meat. The positive association between pH_{24} and meat redness following 35 days storage, and the failure of vitamin E to influence this association, suggests that the premise that increasing pH_{24} would increase free radical production and thus myoglobin oxidation was incorrect.

Vitamin E supplementation did however have a greater impact on the display colour of short-stored loin meat with a high IMF content, in line with our hypothesis. Increasing muscle vitamin E content improved meat colour throughout the 72 hour display in high IMF short-stored meat. Increasing intramuscular vitamin E therefore substantially improved the initial bloomed colour of the meat before the time when metmyoglobin or browning are anticipated to negatively impact meat colour (Fig. 7-6). Less is known of how intramuscular vitamin E influences bloomed meat colour as opposed to retail colour stability or browning, thus it is difficult to speculate how vitamin E may be influencing myoglobin oxygenation dynamics and thereby bloomed colour in high IMF meat. This is nonetheless a promising finding given current industry selection for high IMF and the importance of bloomed colour to consumer appeal of lamb meat on retail display.

7.5 Conclusion

Dietary vitamin E supplementation was not able to mitigate the negative influence of long storage on the colour stability of lamb meat. However, dietary vitamin E supplementation did improve the colour stability of lamb loin on retail display regardless of meat storage time. Given that 70 days is considered close to the upper limit for chilled lamb storage prior to retail display, and that vitamin E had maximal impact on the colour stability of meat stored for 35 days, it is likely that vitamin E supplementation can improve the retail colour of fresh stored lamb products. Muscle ICDH activity and pH_{24} did not influence the effect of vitamin E on meat colour stability, though the impact of increased vitamin E concentration was amplified in high IMF short-stored lamb meat. This is a promising result suggesting that vitamin E supplementation can negate the negative influence of current industry efforts to improve taste qualities by increasing IMF content lamb meat. Therefore, while vitamin E supplementation may be more beneficial in short-stored lamb product marketed domestically than in product marketed overseas after extended shipping, dietary vitamin E supplementation will improve the colour stability of lamb loin meat following chilled storage for up to 70 days.

Acknowledgements

The authors gratefully acknowledge the financial support of the Cooperative Research Council for Sheep Industry Innovation. The authors also wish to thank the people that assisted with the management of the lambs at Great Southern Research Station and to those that assisted with sample collection and measurement as well as abattoir staff.

Chapter 8. General Discussion

8.1 The benefits of improving meat colour to the lamb meat industry

The colour of lamb meat is important to the Australian lamb meat industry given it is crucial to consumer appeal and purchase. Reducing lamb meat discolouration will reduce downgrading and extend the colour shelf life of lamb meat, thereby improving the profitability and efficiency of the lamb meat supply-chain. Though it is difficult to quantify the cost of meat discolouration in Australia without a grading system for meat colour in place, in the US an average of 3.7% of red meat sales are estimated to be lost due to colour deterioration (Williams, Frye et al. 1992). If applied to the Australian sheep meat industry this rate of lost sales would equate to an annual loss of AUD $145 million for the industry. Though certain factors influencing meat colour such as ambient temperature are well understood and tightly controlled along the lamb meat supply chain, meat discolouration continues to be an important limitation to the marketability of fresh lamb meat. The lamb meat industry therefore recognises that better understanding of meat colour is needed to develop more successful strategies for reducing discolouration across the supply chain. Understanding the genetic, production, processing and retail factors influencing meat colour is critical to developing practical solutions to these industry problems.

8.2 Bloomed lamb loin colour and its stability over retail display in the information nucleus flock experiment

The INF experiment provides a comprehensive assessment of bloomed lamb loin colour and its stability over retail display under Australian production conditions. This experiment therefore provides valuable insights into meat colour problems that are likely to affect the lamb industry as a whole. This extensive study suggests that insufficient meat lightening with blooming and the rapid browning of wrapped lamb on retail display are the key meat colour problems limiting the value of lamb meat on retail display.

The bloomed colour of lamb meat has been poorly described in the literature to date. Thresholds of consumer acceptability of bloomed meat L^* and a^* have been established though the ideal L^* and a^* values for lamb meat remains unclear. The acceptable or ideal levels of b^*, hue angle and chroma in red meats are unknown. There is also a lack of standardised methodology in the measurement of bloomed meat colour between research publications. Meat surface L^*, a^* and b^* values can be measured using colorimetric or spectrophotometric instruments; however the colour values produced by different instruments are not directly comparable. Instrument specifications such as aperture size or degree of observer may also influence L^*, a^* and b^* measures, meaning that direct comparisons between meat colour studies may be unreliable when different instrument specifications have been used. The extensive data set captured via consistent colorimeter specifications and analysed in Chapter 3 therefore provides a valuable resource as a benchmark of current ranges in L^*, a^*, b^*, hue angle and chroma of lamb loin meat from different breeds in Australia.

The amount of lamb meat currently discounted or rejected by consumers at retail due to poor bloomed colour is unclear. Bloomed meat colour (*L**, *a**, *b**, hue angle and chroma) was measured in over 8000 mixed breed lambs in the INF experiment and thereby provides a gauge of meat colour problems affecting the Australian industry. Every meat sample in this study had an *a** value greater than 9.5 and therefore would be deemed acceptable by the average consumer (Khliji, Van de Ven et al. 2010). Meat *a** measures have the highest correlation with consumer acceptance of meat colour compared to other measures of meat colour including R630/R580 (Khliji, Van de Ven et al. 2010).

Meat *L** is also important for consumer acceptability of meat colour as consumers associate dark meat with reduced meat quality. However, only 60% of loin samples in the INF experiment exceeded the threshold of 34 required for consumer acceptance of lamb meat lightness. This supports anecdotal reports that dark meat is the most important discolouration problem causing consumer rejection of freshly cut lamb meat. Industry efforts to improve consumer acceptance of lamb meat colour therefore need to focus on increasing meat *L**. This could be achieved by increasing selection for muscle or by reducing the age of lambs at slaughter, and thereby reducing the myoglobin content of lamb meat, or by continued selection for increased intramuscular fat concentrations in lamb meat. The importance of changes in meat *b**, hue angle and chroma reported in this study are difficult to determine given the scarcity of information available on their relationship with consumer acceptance of meat colour.

The colour stability of bloomed lamb loin was measured over a 3 day simulated display in a subset of the INF lambs (n = 4404). Meat redness was measured every 24 hours over the display using R630/R580 rather than *a**, as spectrophotometric measures

provide an estimate of the changes in myoglobin pigment forms with time on retail display. Bloomed lamb loin had an average R630/R580 of 5.38 at the start of display, which reduced to 4.22, 3.52 and 3.05 after 24, 48 and 72 hours on simulated retail display. Khliji et al. (2010) reported that R630/R580 greater than 3.5 is needed for consumer acceptance of lamb loin colour, meaning that more than 55% of lamb loins from the INF experiment were too brown for consumer acceptance after only 48 hours on retail display. After 72 hours, consumers would have rejected more than 75% of the lamb loins due to browning. These results confirm that poor colour stability substantially limits the retail colour shelf life of wrapped lamb meat in Australia. Despite the substantial amount of research invested into red meat colour stability, better strategies are required to reduce the rapid browning of overwrapped lamb meat.

8.3 The influence of production traits on the colour of lamb meat during retail display

Production factors including lamb slaughter group, site and year of production had more substantial effects on lamb loin colour than any intrinsic muscle factors measured in this study, contrary to expectations. Accounting for muscle traits known to impact meat colour such as myoglobin or pH_{24} did not account for these production effects on meat colour. Further investigation is therefore required to understand how these factors are influencing bloomed meat colour and its stability over retail display. A multitude of interacting factors including animal genetics, management practices, nutritional differences and environmental factors are likely to be impacting muscle biochemistry and subsequent meat colour independent to the carcass and muscle traits measured in this study.

Slaughter group

The grouping of lambs sent for slaughter was associated with greater magnitude differences in meat *L**, *a**, chroma and R630/R580 during simulated display than all other production, carcass and muscle traits. The effect of lamb slaughter group on meat *b** and hue angle was second in magnitude only to the site of lamb production. The effect of slaughter group was substantial despite slaughter groups having been compared only within any one site and year of production. Slaughter group nonetheless likely captures a number of different factors that may indirectly influence lamb meat colour such as environmental conditions on the day of slaughter; transport and lairage conditions, and in management practices and nutrition leading up to slaughter, given that these groups of lambs were slaughtered during different seasons of the year.

No patterns in lamb meat colour could be seen between lamb slaughter groups, either within a particular site, between sites or across each year. While the design of this experiment limits our ability to analyse these effects further, we can speculate on possible mechanisms. The nutritional history of lambs in this study were complex, including differences in the number and variety of pastures and the type of supplementary feed provided at different sites, at different times of year and in different years of production (Ponnampalam, Butler et al. 2014). Muscle traits such as vitamin E concentration that influence meat colour and are determined by nutritional intake would have varied dramatically between seasons and thus between slaughter groups as well as between site and years. Dietary antioxidant intake is therefore likely to account for some of the variation in meat colour observed between lamb slaughter groups.

Site of Production

The site of lamb production also had substantial effects on lamb meat colour. The site effect likely captured substantial differences in nutrition, in on-farm production routines and in processing at different abattoirs. The site of lamb production would also reflect differences in lamb nutrition leading up to slaughter even greater than those seen between slaughter groups or years of production, given that the sites of lamb production in this study were up to 3500 km apart and thus had markedly different climates. For example, Katanning in Western Australia has an elevation of 320m and an average yearly rainfall of 455 mm, while Kirby in New South Wales has an elevation of 1079m and receives 720 mm of rain annually. Given the climatic variation and vast distances between sites in this study, there would have been substantial variation in the type and availability of pastures and in the type and need for supplementary feed. The vast difference in nutrition between sites, seasons and years in this study underpins the large range in lamb ages in this study (370 days). Lambs were assigned to groups to achieve a set carcass weight at slaughter, meaning that the growth rate or the time taken for lambs to reach slaughter weight determined the ages of lambs at slaughter.

The impact of site was particularly large on fresh meat *b** and therefore hue angle and chroma, suggesting that the type of nutrition fed to lambs prior to slaughter may be a particularly important determinant of meat *b**. The nutritional influence on meat *b** may relate to the yellowness of fat being primary determined by dietary intake of carotenoids (Daley, Abbott et al. 2010), which substantially varies within and between feed types. Alternatively, an unidentified nutritional component may be influencing the perceived *b** of muscle tissue itself. The industry significance of this finding is difficult to gauge given that the importance of *b** to consumer acceptance of meat colour is unknown.

In addition to nutritional variation, factors such as the transport distance to abattoirs also varied substantially between sites. Each production site sent lambs a set distance to an abattoir, though some abattoirs received lambs from more than one production site. Site management factors and transport distances influence the stress ensured by lambs prior to slaughter, which influences muscle traits and may therefore influence meat colour. Stress prior to slaughter is most commonly linked to changes in muscle pH, though variation in pH_{24} did not account for the impact of site in this study. Animal stress or dehydration prior to slaughter may influence other muscle factors such as its physical structure, and thereby influence meat colour.

Year of production

The year of lamb production was associated with less variation in lamb loin colour than site or slaughter group. This is unsurprising given that less variation in nutritional, genetic and management practices is expected between years of production than between diferent sites and seasons. Though climatic conditions may vary from year to year, pasture and supplementary feed types will vary much less between years than between different sites. Animal management practices are also likely to differ less between years given that farm managers were consistent between years though not between sites.

Breed type and sex

The impact of lamb breed and sex on loin meat colour were small compared to other production and muscle factors. Lamb sire type influenced meat *L**, Terminal sired lambs producing the lightest meat and Merinos the darkest. These breed type effects were underpinned by changes in muscle myoglobin, iron and ICDH activity rather than

by changes in meat pH_{24}. This suggests that increased oxidative capacity/and or myoglobin concentration underpins the darker meat colour from Merino sired lambs, contrary to the perception that Merinos produce darker meat due to their propensity to produce high pH meat (Gardner, Pethick et al. 2006). Female lambs produced marginally darker meat than males, a difference that could not be explained by carcass or muscle factors. Teixeira et al. (2005) also found that female lambs produced darker loin meat, though reported a far larger magnitude of effect, which may be due to the low number of lambs (n = 72) in that study.

Lamb breed and sex had little effect on the bloomed meat *a**, *b**, hue angle and chroma compared to other factors and previously reported effects (Hopkins and Fogarty 1998, Teixeira, Batista et al. 2005). Maternal sired lambs produced meat with higher *a**, *b**, hue angle and chroma than Merino or Terminal sired lambs, though no carcass or muscle trait could account for this difference. Maternal dams produced higher meat *a** and female lambs higher meat chroma, though these effects could be attributed to higher myoglobin concentration.

Lamb breed influenced meat colour stability over simulated display though sex did not. Terminal sired lambs produced the highest R630/R580 after 72 hours display, followed by Maternal and Merino sired lambs. Merino dams also produced the lowest R630/R580 meat at the end of the simulated display, meaning that pure Merino lambs produced the darkest bloomed meat colour with the poorest colour stability in this study. These breed effects on colour stability were accounted for by changes in ICDH activity, supporting the hypothesis that greater muscling potential in Terminal and Maternal lambs increases glycolytic muscle fibres (Hawkins, Moody et al. 1985, Greenwood, Gardner et al. 2006,

Greenwood, Harden et al. 2007) thereby reducing muscle oxidative capacity and improving meat colour stability.

Lamb age

Lamb age strongly influenced lamb meat colour in the INF experiment. Increasing lamb age from 140 to 400 days markedly reduced bloomed meat *L**. This effect was reduced by two-thirds when myoglobin and iron concentrations were accounted for, though did not alter when ICDH activity or pH_{24} were accounted for. This suggests that the impact of lamb age on bloomed meat *L** relates to increasing muscle myoglobin and iron content with age (Kelman, Pannier et al. 2014, Pannier, Pethick et al. 2014) rather than to changes in muscle oxidative capacity or meat pH.

In line with the impact of increasing myoglobin and iron concentration, meat *a**, *b**, hue angle and chroma also markedly increased with lamb age. These effects were less than half the magnitude of effect of lamb age on meat *L**, aligning with the greater impact of myoglobin and iron on meat *L** compared to *a** and chroma. While the increase in meat *a** and chroma with increasing lamb age is positive, this benefit is offset by increased meat *b** and hue angle and particularly reduced meat *L**.

Though increasing lamb age improved bloomed meat redness (*a**), meat from older lambs had reduced colour stability on retail display. Lamb age increased R630/R580 at the start of display, likely due to the positive associations between lamb age, myoglobin concentration and bloomed meat redness. However, the impact of lamb age on R630/R580 reduced with time over display to the point that increasing lamb age reduced meat R630/R580 after 72 hours display. The increased browning of meat from older lambs at 72 hours display is likely due to the association between lamb age and

muscle oxidative capacity. As myoglobin concentration and muscle oxidative capacity increase with age, older lambs produce darker meat that browns faster on retail display and that is more likely to be rejected by consumers.

8.4 The influence of carcass and muscle traits on the colour of lamb meat during retail display

Muscle traits such as pH_{24}, myoglobin concentration, and IMF content are associated with key biochemical processes underpinning meat colour and therefore directly influence bloomed colour and its stability on retail display. Understanding the influence of carcass and muscle traits on lamb meat colour is important, as industry has greater capacity to manipulate or control these traits to improve meat colour.

pH_{24}

Loin pH_{24} had pronounced and consistent effects on lamb meat colour. Increasing pH_{24} of the loin muscle from 5.4 to 6 reduced meat *L**, *a**, *b**, hue angle, chroma and R630/R580 over the simulated retail display. The substantial effects of pH on lamb loin colour are unsurprising given the importance of pH in the biochemical processes governing meat colour: oxygen penetration, *post-mortem* metabolism and myoglobin oxidation. Given the interrelated nature of these processes, determining the precise mechanism/s underpinning the effects of pH_{24} on bloomed meat colour and its stability on retail display is difficult.

Increasing pH_{24} was associated with substantial reductions in meat *L**, supporting industry perception and the majority of literature citing high ultimate pH as a key cause of dark red meat (Lawrie 1983, Faustman and Cassens 1990). High meat pH is thought

to darken the perceived colour of red meats by altering the physical structure of meat, thereby obstructing oxygen diffusion and increasing light absorption in meat (Andrés-Bello, Barreto-Palacios et al. 2013). A high pH also favours *post-mortem* oxidative metabolism, which outcompetes myoglobin for oxygen, reduces the proportion of oxymyoglobin: deoxymyoglobin and thus darkens the meat surface. However contrary to our hypothesis of Chapter 3, muscle myoglobin concentration had a greater effect on lamb loin lightness than pH_{24}. Also unexpectedly, changes in loin pH_{24} did not account for any production effects on meat *L**.

Changes in loin pH_{24} had the greatest magnitude impact on bloomed meat *a**, which has been reported to be the single best indicator of consumer acceptability of lamb meat colour (Khliji, van de Ven, Lamb, Lanza, and Hopkins, 2010). Changes in loin pH_{24} also produced the largest magnitude effect on chroma, another important parameter in the evaluation of meat colour by consumers (AMSA 2012). The effect of pH_{24} on meat *a** and chroma is likely underpinned by the reduction in bloom depth and oxymyoglobin: deoxymyoglobin in high pH meat. Increasing pH_{24} also reduced *b** and hue angle of loin meat, meaning a redder hue and less yellow meat colour. These changes are likely beneficial in terms of consumer appeal, however a redder hue in high pH_{24} meat conflicts with reduced loin *a** and R630/580 in high pH_{24} meat in this study. *a** and R630/R580 are well established and widely used measures of meat surface redness as opposed to hue angle values, thus overall pH_{24} substantially reduces meat redness formed with blooming and its stability across retail display in this study.

The substantially reduced colour stability of lamb loin meat with increasing pH_{24} counters various studies reporting that high pH improves the colour stability of red meat as low pH favours metmyoglobin formation(AMSA 2012). However, in the 4404 lamb

loins assessed in this study, increasing pH from 5.4 to 6.0 substantially reduced R630/R580 throughout the 72 hour simulated retail display. The increased browning in high pH meat may be attributable to a) reduced oxygen penetration and thus bloom depth, b) increased oxygen consumption increasing the proportion of deoxymyoglobin which is less stable than oxymyoglobin and thereby more prone to oxidation; and/or c) increased metabolic activity increasing oxidative free radicals (AMSA 2012). The mechanisms through which pH_{24} influences bloomed meat colour and colour stability are therefore similar though distinct. The consistent negative impact of pH_{24} on bloomed meat lightness, redness and colour stability in this study clearly demonstrates that reducing the ultimate pH of lamb meat should be a key focus of the lamb meat industry to reduce losses caused by meat discolouration.

Myoglobin concentration

The myoglobin concentration of the loin muscle had important influences on bloomed lamb colour though only small effects on colour stability. Myoglobin concentration had inconsistent effects on bloomed meat colour, in contrast to the effects of pH_{24}. Increasing myoglobin from 4 to 12 mg/g of muscle substantially reduced meat *L**; moderately increased *a** and chroma; and slightly decreased loin *b** and hue angle. Reduced *L** in high myoglobin meat is likely caused by myoglobin pigments absorbing light and thereby reducing light reflected from the meat surface, and possibly by an associated increase in oxidative muscle capacity and oxygen consumption (AMSA 2012). The increase in *a** and chroma and decrease in *b** and hue angle with increasing myoglobin are all positive effects. However, given that changes in myoglobin had a greater magnitude impact on meat *L** than any other intrinsic muscle factor, and that meat darkening is the most important bloomed colour problem in this study, the

negative impact of increasing myoglobin on meat *L** outweighs its positive effects on bloomed colour.

The greater impact of myoglobin compared to pH_{24} on meat *L** contradicts our hypothesis and common industry perceptions that the ultimate pH of meat is the most important factor causing dark red meat. This perception underpins the use of ultimate pH by the beef industry as a rough gauge of bloomed meat colour. Meat pH could have higher relative importance where lambs produce meat with ultimate pH levels over 6 due to poor nutrition and/or stress, though the lambs evaluated in this study are a good reflection of current Australian production systems. Therefore this study demonstrates that myoglobin is as a more important determinant of lamb meat *L** than pH and may be a better predictor of dark cutting lamb meat.

Myoglobin concentration had a strong influence on bloomed meat *a** and a moderate influence on R630/R580, which reduced with time over display. The increased meat *a**, reduced hue angle and increased chroma in high myoglobin meat is underpinned by an increased density of oxymyoglobin in the meat surface. Increased oxymyoglobin density in high myoglobin meat likely also underpins the substantial increase in R630/R580 at the start of display. While myoglobin concentration was positively associated with all R630/R580 measures across display, the magnitude of its impact reduced from 1.37 units to only 0.14 units of R630/R580 across the display period. When HCWT was accounted for, increasing myoglobin had a small negative impact on R630/R580 at 72 hours display. While increased myoglobin concentration was hypothesised to worsen lamb meat colour stability due to its association with muscle oxidative capacity, the impact of myoglobin on bloomed colour may have obscured this effect. The substantial effect of myoglobin on R630/R580 at 0 hours likely reflects the

positive influence of myoglobin on bloomed meat redness. The reduced impact of myoglobin on R630/R580 with time on display may then relate to the positive association between myoglobin and muscle oxidative capacity, which is linked to increased lamb meat browning (O'Keeffe and Hood 1982, McKenna, Mies et al. 2005, Calnan, Jacob et al. 2014).This interpretation is supported by the negative impact of increasing iron concentration and ICDH activity on R630/R580 after 72 hours display. Regardless, the impact of increasing myoglobin concentration on retail meat browning appears minimal.

The conflicting influences of myoglobin concentration on bloomed colour and its stability on retail display must be considered to provide useful recommendations to industry. The improvement in bloomed meat *a** with increasing myoglobin concentration is countered by the substantial reduction in meat *L**. However, this study shows that dark meat is a greater problem in the Australian lamb meat industry than meat redness, supporting anecdotal reports that dark meat most frequently causes consumer rejection of freshly cut lamb meat. Therefore increasing meat *L** by reducing muscle myoglobin and pH_{24} should be a key focus for the lamb meat industry. The high genetic correlation reported between myoglobin and lamb loin *L** of -0.81 (Mortimer, van der Werf et al. 2014) suggests that sire selection may successfully lower myoglobin and thus reduce dark lamb meat. Genetic selection for increased muscling and thus reduced oxidative muscle fibres, combined with slaughtering lambs at a younger age, may also reduce the myoglobin concentration and increase lamb meat *L** (Kelman, Pannier et al. 2014). Reducing muscle myoglobin concentration may have the additional benefit of reducing the rate of lamb meat browning on retail display.

Iron concentration

Given that iron is an essential component of myoglobin, muscle iron concentrations had similar effects on lamb meat colour to myoglobin. Increasing iron concentration substantially reduced meat *L**, increased *a** and chroma, and decreased meat *b** and hue angle. The magnitudes of these effects were slightly less than with myoglobin, meat *L** reducing by 7% less with increasing iron than myoglobin. The impact of iron concentration on lamb meat colour is likely to be largely underpinned by associated changes in myoglobin concentration.

High iron content also increased R630/R580 at the start of the simulated display. Like myoglobin, this effect likely relates to the crucial role of the iron-containing myoglobin in bloomed meat redness, rather than an effect on meat browning. Iron was not associated with R630/R580 at 24 or 48 hours of display, and in contrast to myoglobin, was negatively associated with R630/R580 after 72 hours display. The negative influence of iron content on meat redness at the end of display likely reflects the negative impact of muscle oxidative capacity on colour stability, as ROS by-products increase with time after blooming and thus may only express their impact on meat colour later in the display period. Iron is central to haemoglobin and myoglobin pigments, which are both more abundant in oxidative muscle fibres, therefore iron may be more closely associated to muscle oxidative capacity than myoglobin concentration.

Isocitrate dehydrogenase (ICDH) activity

As an essential enzyme in the oxygen-dependant citric acid cycle of mitochondria, ICDH activity has been used as a marker of oxidative capacity in lamb muscle (Gardner, Pethick et al. 2006). Muscle ICDH activity had similar effects on bloomed

meat colour as myoglobin and iron concentrations, though ICDH activity consistently worsened colour stability from 24 hours of display. The substantial and consistent impact of ICDH activity on R630/R580 from 24 to 72 hours of display aligns with our hypothesis that ICDH activity reflects muscle oxidative capacity, which reduces the colour stability of lamb meat. Unlike myoglobin and iron concentration, ICDH activity was not associated with R630/R580 at 0 hours of display, supporting that the effect of ICDH activity on meat colour is not simply a reflection of meat pigmentation.

ICDH activity had small influences on bloomed meat colour. Increasing ICDH activity reduced meat *L** and increased meat *a** by only half the magnitude of myoglobin, supporting that the strong associations between myoglobin and meat *L** and *a** relate to changes in pigment density rather than to changes in muscle oxidative metabolism. Additionally, the magnitude impact of myoglobin on meat *L** reduced by only 5% when changes in ICDH activity were accounted for. Overall, these results suggest that reducing muscle oxidative capacity will improve the colour stability of lamb meat on display and will have mixed influences on bloomed meat colour associated with changes in myoglobin content.

Intramuscular fat (IMF) concentration

IMF concentration was hypothesised to have similar effects on lamb meat colour to myoglobin, iron and ICDH activity due to the link between IMF and muscle oxidative capacity (Hocquette, Jurie et al. 2003). Contrary to these expectations, increasing IMF from 2-8% increased meat *L**substantially, IMF having a greater magnitude effect on meat *L** than myoglobin or pH_{24}. Additionally, the effect of IMF on meat *L** was reduced by < 10% when myoglobin or iron were accounted for, suggesting that IMF influences meat *L** independent to associated changes in muscle oxidative capacity. The

perceived lightness of highly marbled meat therefore likely relates to increased light reflection from the white fat in the meat surface. This positive association could be of substantial benefit to the industry given the importance of meat lightness and IMF content to the visual appeal and taste of lamb meat.

Increasing IMF also increased meat *a**, *b**, hue angle and chroma, in line with the effects of myoglobin, iron and ICDH activity. IMF had a substantial effect on meat *a**, of the same magnitude as myoglobin concentration. However the magnitude of this effect was reduced by only 10% when myoglobin content was accounted for, demonstrating that associated changes in myoglobin do not underpin the improved bloomed meat *a** in high IMF meat. Changes in pH_{24} did not account for this effect either, leaving the key mechanism underpinning this advantageous association unclear.

The positive effects of IMF on bloomed meat colour are very positive for the Australian industry given current selection for increased IMF to improve taste qualities of lamb meat. However, these positive effects are off-set by a reduction in meat colour stability with increasing IMF. In line with expectations, increasing IMF reduced R630/R580 throughout the simulated retail display. This effect is thought to relate to associated increases in lipid peroxidation, which triggers myoglobin oxidation and thereby increases meat browning (Faustman, Sun et al. 2010). The magnitude impact of IMF on R630/R580 across display was small, however compared to other muscle factors including pH_{24}, ICDH activity, HCWT and relative shortloin muscle or fat weight. Therefore, the negative effect of increasing IMF on meat colour stability may be minimised by controlling factors such as pH_{24} or using methods such as MAP.

Shortloin muscle weight

Increasing shortloin muscle weight adjusted for whole carcass weight, reflecting greater phenotypic muscularity, had moderate negative effects on bloomed meat colour, though substantial positive effects on meat colour stability. Adjusted shortloin muscle weight can be considered a phenotypic expression of genetic selection for increased muscling via sire PEMD estimates, and may therefore be associated with reduced muscle oxidative capacity (Greenwood, Gardner et al. 2006, Gardner, Williams et al. 2010). However, reduced meat *L** with increased adjusted shortloin muscle weight does not support this hypothesis. The negative association between adjusted shortloin muscle weight and bloomed meat colour instead appears to be caused by an associated reduction in IMF in the loin muscle. When IMF was accounted for, adjusted shortloin muscle weight was positively associated with meat *L**. IMF also accounted for half the magnitude impact of adjusted shortloin muscle weight on meat *a**, suggesting that a combined reduction in IMF and myoglobin may be driving this effect. A reduction in IMF may also account for the positive effects of increasing adjusted shortloin muscle weight on R630/R580 across the simulated retail display. However the impact of shortloin muscle weight on R630/R580 are of far greater magnitude than the impacts of IMF, thus other unknown factors must be contributing to the positive effect of loin muscle weight on retail colour stability.

Shortloin fat weight

Increasing shortloin fat weight adjusted for whole carcass weight increased meat *L**, *a**, *b**, hue angle, chroma and R630/R580 across the simulated retail display. Increasing adjusted shortloin fat weight from 100 to 500g had a greater magnitude of impact on

bloomed meat colour than increasing adjusted shortloin muscle over the same weight range, though not on colour stability over display.

These impacts of shortloin fat on lamb meat colour are largely negative considering that the lamb industry aims to reduce shortloin fat weight to improve lean meat yield. However, the effects of reducing shortloin fat on bloomed meat colour also appear to be underpinned by an associated reduction in IMF. The negative effect of reducing shortloin fat weight on meat *L** and *a** reduced by 89% and 39% when IMF was accounted for. Therefore, if the lamb industry can independently select to maintain or increase IMF levels at the same time as reducing shortloin fat, then any negative influence of reducing carcase fatness on bloomed meat colour, particularly meat *L**, will be minimal.

The negative impact of reducing shortloin fat weight on meat colour stability cannot be attributed to changes in IMF, nor to changes in muscle oxidative capacity. This counters the expectation that reducing shortloin fat weight is linked to reduced muscle oxidative capacity, given that shortloin fat weight is considered a phenotypic marker of sire PFAT estimates and reduced PFAT is associated with reduced muscle oxidative capacity (Kelman, Pannier et al. 2014). The mechanism causing reduced shortloin fat weight to reduce the colour stability of lamb loin therefore remains unclear.

Hot Carcass Weight (HCWT)

Substantial variation in lamb HCWT was observed in the INF experiment despite lambs being grouped for slaughter based on a set target carcase weight. Increasing lamb HCWT from 15 to 35 kg had substantial positive effects on loin colour, increasing meat *L**, *a**, *b**, hue angle, chroma and R630/R580 across the simulated retail display.

Increasing HCWT only slightly influenced meat *L**, though substantially influenced meat *a** and R630/R580. In this study increasing HCWT represents increased lamb growth rate, given that slaughter groups were accounted for in the statistical models and that lambs within slaughter groups were of similar ages. Increased R630/R580 with increasing HCWT thereby supports our hypothesis that increased growth rate would improve meat colour stability. This hypothesis was based on an anticipated reduction in muscle oxidative capacity with increased growth rate/ HCWT, however the effects of HCWT on meat colour did not change when indicators of oxidative metabolism were accounted for. Furthermore the effects of HCWT on meat colour could not be accounted for by changes in any muscle factor measured in this study. Nor did accounting for HCWT substantially change the influence of other muscle factors on lamb meat colour in this study. Therefore, the mechanisms underpinning the substantial positive effects of increasing HCWT on lamb meat colour remain unclear.

Zinc concentration

The zinc concentration of lamb muscle had small influences on bloomed meat colour, reducing *L** and slightly increasing *a**, *b** chroma and hue angle, in line with the effects of myoglobin and iron concentration. Zinc concentration did not influence meat colour stability. These results are positive in that industry selection for high zinc levels to improve the human health benefits of lamb meat will not worsen meat colour.

8.5 The influence of selection for lean meat yield using Australian Sheep Breeding Values on the colour of lamb meat during retail display

Australian sheep breeding values (ASBVs) for post-weaning weight (PWT), post-weaning eye muscle depth (PEMD) and post-weaning c-site fat depth (PFAT) had largely positive or neutral influences on lamb loin colour. Increasing PEMD was associated with increased bloomed meat *L**, *a**, *b**, hue angle and chroma in one or more breed and sex type combinations, however did not have a significant effect in the majority of lambs, and even reduced meat *a** and chroma in one breed type grouping. The variable effects of PEMD on bloomed colour between breed types likely relates to the inconsistent effect of PEMD on myoglobin concentration between breed types reported by Kelman et al. (2014). The positive association between PEMD and meat *L** became consistent across breed types when myoglobin concentration was accounted for. Changes in ICDH activity and myoglobin could account for some PEMD effects on meat *a**, however, no muscle or carcass traits could account for the effect of PEMD on meat *L**, leaving the cause of this positive relationship unknown.

Selection for high PEMD also had a positive influence on meat colour stability. Increasing PEMD moderately increased loin R630/R580 after 72 hours display. The impact of PEMD on R630/R580 was reduced when changes in ICDH activity were accounted for; supporting the hypothesis that selection for muscling in lambs via increasing PEMD improves colour stability via reduced muscle oxidative capacity. The substantial impact of PEMD on R630/R580 at the end of display is larger than anticipated, given the marginal reductions in ICDH activity and myoglobin

concentration with selection for PEMD reported by Kelman et al (2014). It is likely that selection for high PEMD sires is altering other unidentified factors in lamb muscle that are also influencing meat colour traits such as *L** and R630/R580 on display.

Increasing lean meat yield via selection for low PFAT sire breeding values had mixed effects on bloomed meat colour. The increased meat *L** with reducing PFAT sire estimates could be attributed to changes in muscle myoglobin and iron concentrations, supporting the hypothesis that PFAT influences meat *L** due to associated changes in muscle oxidative capacity rather than due to changes in IMF. Reducing PFAT had small and inconsistent effects on meat *a**, *b** and chroma, and no effect on hue angle. Reduced myoglobin and iron content with reducing PFAT accounted for the effect of PFAT on meat *a**. The reason for the inconsistent effects of PFAT on meat *a**, *b** and chroma between lamb breed types is however difficult to explain, given that Kelman et al. (2014) reported reduced myoglobin in lamb loin with selection for low PFAT regardless of breed type.

Increasing lamb growth rates via high sire PWT estimates did not influence lamb meat colour. PWT sire estimates were expected to influence the display colour of lamb meat as high PWT sires produce faster growing lambs that are less mature at slaughter (Hall 2000) and thus have reduced muscle oxidative capacity (Suzuki and Cassens 1983). However, increasing PWT was associated with increased ICDH activity and myoglobin concentration in the study from Kelman et al. (2014). The cause of this apparent increase in muscle oxidative capacity with selection for high PWT is unclear. Regardless, the lack of association between PWT and meat colour in this study suggests that changes in muscle myoglobin or oxidative capacity with selection for PWT are not substantial enough to influence meat colour.

Overall, these are important and promising findings for the lamb industry demonstrating that genetic selection for lean meat yield will have neutral or positive effects on the display colour of lamb meat. While selection for high PEMD sires and low PFAT sires had mixed effects on bloomed meat colour, the improvement in meat L^* is important given the current problems with dark meat in the industry. Selection for PEMD will also improve the colour stability of lamb meat, another important limitation to retail sale of lamb meat, while the lack of association between PWT and loin colour is positive for industry as it suggests that continued selection for increased lamb growth rates will not have detrimental effects on meat colour.

8.6 Using bloomed colour to predict the colour stability of lamb meat on retail display

Meat redness (R630/R580) at the start of retail display could not accurately predict of lamb meat colour after 3 days of simulated retail display in this study. Additionally, accounting for meat pH_{24}, IMF concentration and lamb age did not improve the predictive ability of initial R630/R580. General linear model associations between R630/R580 at 0 hours display and subsequent measures described only 10% of the variation in colour and did not considerably improve with adjustment for lamb production effects and carcass covariates such as age, pH_{24} and IMF. These results suggest there is little capacity to use bloomed meat colour measured at the start of retail display to predict meat browning on subsequent days of display, even with considerable information on animal background and carcass traits.

The low correlation between R630/R580 at 0 and 72 hours display suggests that R630/R580 at 0 hours represents bloomed meat colour, which is a separate trait of little

use in predicting meat colour stability on retail display. Reflectance measures such as R630/R580 provides information on the chemical myoglobin forms present in a meat surface and are therefore most commonly used to measure meat browning or metmyoglobin accumulation over retail display. However, R630/R580 at 0 hours was measured immediately after the meat was bloomed for 30 - 60 minutes, before any substantial quantity of metmyoglobin would have accumulated. The substantially different impact of muscle factors such as myoglobin concentration on R630/R80 at 0 hours and subsequent R630/R580 over retail display supports this interpretation.

In contrast, meat colour measured at 24 hours display can accurately predict meat browning at 2 to 3 days of retail display. Lamb loin R630/R580 were highly correlated at 24, 48 and 72 hours of display, R630/R580 at 24 hours describing 67% of the variation in R630/R580 at 72 hours. When carcass and muscle traits were accounted for, R630/R580 at 24 hours described 72% of the variation in R630/R580 after 72 hours on display. These results suggest that meat colour measured at 24 hours of retail display could provide an excellent prediction of meat browning in subsequent days of retail display, without the need for any animal information at retail. However, measuring meat colour after 24 hours of display is not practical in a retail setting, therefore the value of R630/R580 may be limited to the use of measures at 24 hours display to drive genetic improvement of colour stability via the development of a retail colour breeding value for lamb meat.

8.7 The use of dietary vitamin E supplementation to extend the redness of highly marbled long-aged lamb loin on retail display

Increasing intramuscular vitamin E concentration via dietary supplementation successfully increased the colour stability of lamb loins following 5, 35 and 70 days of chilled storage. However, contrary to our hypothesis, vitamin E supplementation did not have a greater effect on colour stability in longer aged meat. Instead, increasing vitamin E concentration had a similar magnitude impact on R630/R580 at the end of display regardless of storage time prior to retail display. The failure of vitamin E to have a greater effect in long-stored meat may be due to a) a reduced concentration or efficacy of vitamin E with extended storage; b) a high oxidative load in long-stored meat overwhelming the antioxidant capacity of vitamin E; or c) a failure of oxidative load to increase with meat storage time.

Vitamin E supplementation had a greater impact on the colour stability of loin meat with a high IMF content. The substantial improvement in colour stability of high IMF short-stored lamb meat following vitamin E supplementation is a promising finding for the industry, suggesting that vitamin E supplementation may be increasingly important as the industry selects for increased IMF to improve the taste qualities of lamb meat. However, IMF was associated with colour stability only in short-stored lamb meat. Though unexpected, the lack of association between IMF and colour in long-stored meat is positive as it suggests that selection for high IMF will not worsen the colour stability of long-stored meat.

ICDH activity was associated with reduced colour stability only in medium- stored meat. The lack of association between ICDH activity and R630/R580 in short-stored meat contrasts with the negative effect of increasing ICDH activity on R630/R580 in the INF experiment (Chapters 5 and 6). Additionally, pH_{24} across a range of 5.49 to 6.11 was not associated with meat colour in short or long-stored lamb meat, and was positively associated with meat redness over display after 35 days of chilled storage. This positive effect of pH_{24} on meat colour contrasts with the strong negative association between pH_{24} and R630/R580 of lamb meat stored for 5 days prior to the simulated display in the INF experiment.

Vitamin E supplementation consistently improved the colour stability of lamb meat in this study regardless of meat storage or muscle factors. Intramuscular vitamin E levels are frequently low in Australian lambs during summer and autumn due to dry conditions, which could have contributed to the poor colour stability of lamb meat seen in the INF. The lamb meat industry therefore needs to consider routine vitamin E supplementation of lambs during dry seasons to improve meat colour on retail display, particularly in the face of selection for increased IMF in lamb meat.

8.8 Limitations and further considerations

The experimental design of the INF limited the capacity to understand what mechanisms may be underpinning some of the effects on lamb meat colour reported in this thesis. The ability to evaluate the effect of lamb age on meat colour was limited in the INF as lamb age was confounded by slaughter group. Lambs within a slaughter group were all of very similar ages due to their production via artificial insemination, slaughter groups having a mean age range of only 11 days. Though prohibiting the

ability to fully separate the effects of lamb age and slaughter group on meat colour, artificial insemination was necessary to evaluate genetic influences on lamb meat colour.

Production effects including site, year of production and slaughter group had substantial effects on bloomed meat colour and its stability over retail display, however no carcase or muscle variables measured in the INF could account for these effects. The reason for the substantial differences between production sites, years and slaughter groups (within site and year) therefore remains unclear. More information on the nutrtition of the lambs prior to slaughter may have improved our understanding of these production effects. For example, differences in vitamin E intake between different sites and seasons may have accounted for some of the substantial differences in meat colour stability observed between sites and slaughter groups. Measuring intramuscular vitamin E concentration in the lambs of the INF would have clarified the contribution of vitamin E to these site and slaughter group differences in meat colour stability. However, measuring intramuscular vitamin E was prohibitively expensive in a data set of this size.

Intramuscular vitamin E concentration was measured in the loin muscle of the 132 lambs in the vitamin E supplementation experiment immediately after slaughter. However, repeating these measurements after 5, 35 and 70 days of chilled storage would have shown if muscle vitamin E concentrations reduced with time in storage. This would potentially explain the reduced the influence of vitamin E on the retail colour stability of lamb meat following extended storage. Additionally, measuring lipid oxidation in the muscle following storage would have demonstrated if lipid oxidation increased in meat during retail display following extended chilled storage, as was hypothesised. Lastly, measuring individual feed intake of the lambs in this study may

have revealed why lambs supplemented with high concentrations of dietary vitamin E produced lower intramuscular vitamin E concentrations than anticipated.

Analyses of additional measures of meat colour over the simulated retail display would also have strengthened the conclusions of this thesis. While R630/R580 is a well established method of measuring colour stability in red meat, additional instrumental colour measures or visual assessments by consumers would have complimented and enhanced the robustness of these findings. Additionally, the use of different instruments to measure bloomed meat colour vs colour stability over the simulated display prevented an effective comparison of meat colour at these time points, as colour measures cannot be directly compared between instruments. Importantly this would have clarified wither meat colour can be measured in the abattoir to predict retail meat colour. Lastly, measuring meat colour more frequently in the first 24 hours of simulated retail display would have determined if meat colour measured between 0 and 24 hours display can accurately predict meat browning over subsequent days of retail display.

8.9 Summary

The INF experiment revealed that 40% of bloomed lamb loins were too dark and over 55% of loins were too brown after 48 hours on retail display for consumer acceptance of meat colour. These results confirm that dark meat and rapid retail browning are substantial problems limiting the marketability of Australian lamb meat and highlights the need for improved strategies to reduce discolouration of lamb meat and the associated losses suffered by the industry.

Production factors including lamb slaughter group, site and year of production had more substantial effects on lamb loin colour than any carcass or muscle factors measured in this study. Contrary to expectations, changes in muscle traits known to impact meat colour such as myoglobin or pH_{24} did not account for any of these production effects on meat colour. Further investigation is therefore warranted to better understand why these production factors are such important determinants of lamb meat colour. The largest differences in meat colour were observed between slaughter groups, despite slaughter groups only being compared within any one site and year of production. The slaughter group effect likely captures the influence of environmental conditions on the day of slaughter; transport and lairage conditions, processing day effects, management practices and nutrition leading up to slaughter.

There were also substantial differences in meat colour observed between the sites of lamb production. This likely results from differences in climate and nutrition between sites, in on-farm production routines and in the abattoirs that processed the lamb carcases from different sites. The year of lamb production influenced meat colour less than site or slaughter group, supporting the notion that less variation in nutrition, genetics and management practices is seen between years at a site than between diferent sites and seasons of production or slaughter groups. Compared to production effects such as site or slaughter group and muscle traits, lamb sex and breed had small effects on meat colour. Some of these effects could be attributed to changes in muscle factors, such as reduced meat lightness in Merino sired lambs due to increased muscle myoglobin content.

Increasing pH_{24} of the loin muscle negatively influenced meat colour; reducing bloomed meat lightness, redness and reducing meat colour stability over retail display.

Myoglobin had a greater magnitude impact on meat lightness than pH, increasing myoglobin markedly reducing meat lightness. However, increasing myoglobin increased bloomed meat redness and had minimal impact on meat colour over retail display. Muscle myoglobin and iron conentrations accounted for the impact of lamb age on bloomed meat color. Increasing IMF substantially increased bloomed meat lightness and redness, IMF having a greater magnitude effect on meat lightness than myoglobin or pH_{24}. These effects of IMF on bloomed meat colour are positive for the Australian industry given current focus on selection for increased IMF to improve taste qualities of lamb meat. The positive effects of increasing IMF are off-set however by a reduction in meat colour stability on retail display.

In Chapter 5 we assessed the ability to predict retail colour stability using bloomed meat colour measured at the start of display and information of lamb age, meat pH and IMF. Bloomed meat colour at the start of retail display was poorly correlated with subsequent meat colour measures over retail dispay. This was still the case when meat pH_{24}, IMF and lamb age were accounted for. Alternatively, meat colour at 24 hours display could accurately predict meat browning at 2 to 3 days of retail display. Measuring meat colour after 24 hours on display is not practical in a retail setting however, thus these measures may only prove valuable in the development of a retail colour breeding value to reduce lamb meat browning on retail display.

Australian sheep breeding values (ASBVs) for post-weaning weight (PWT), post-weaning eye muscle depth (PEMD) and post-weaning c-site fat depth (PFAT) had mostly positive or neutral influences on lamb meat colour. Increasing lamb growth rates via selection for high sire PWT estimates did not influence lamb meat colour, while selection for high sire PEMD increased bloomed meat lightness and redness in some

lamb breed types and improved meat colour stability. Increasing lean meat yield via selection for low PFAT sires increased bloomed meat lightness but had inconsistent effects on bloomed meat redness and did not influence colour stability. Importantly, these results demonstrate that current industry selection for increase lean meat yield will not negatively influence lamb meat colour traits.

High muscle vitamin E improves the colour stability of lamb meat on retail display and is determined by dietary intake. The availability of vitamin E is likely to have differed substantially between sites and seasons of lamb production in the INF experiment and may therefore account for some of the differences in meat colour observed between sites, years and slaughter groups. The efficacy of high muscle vitamin E to improve the colour stability of lamb meat on retail display following extended storage was tested in a dietary supplementation experiment. This work demonstrated that vitamin E improved the colour stability of lamb loin following chilled storage for 5, 35 or 70 days. Contrary to expectations that vitamin E may have an amplified effect in long-stored lamb meat, vitamin E had a similar magnitude impact on meat colour stability regardless of its storage prior to display. Increasing muscle vitamin E did however improve meat colour stability to a greater extent in short-stored lamb meat with high IMF content, suggesting that vitamin E supplementation can negate the negative influence of industry selection for increased IMF on lamb meat colour stability.

References

Adzitey, F. and H. Nurul (2011). "Pale soft exudative (PSE) and dark firm dry (DFD) meats: causes and measures to reduce these incidences-a mini review." International Food Research Journal **18**(1): 11-20.

Agradi, E., G. Abrami, G. Serrini, D. McKenzie, C. Bolis and P. Bronzi (1993). "The role of dietary n-3 fatty acid and vitamin E supplements in growth of sturgeon (Acipenser naccarii)." Comparative Biochemistry and Physiology Part A: Physiology **105**(1): 187-195.

AMSA (2012). AMSA Meat Color Measurement Guidelines. American Meat Science Association. **2nd Edition**.

Anderson, F., D. Pethick and G. Gardner (2016). "The impact of genetics on retail meat value in Australian lamb." Meat science **117**: 147-157.

Anderson, F., A. Williams, L. Pannier, D. Pethick and G. Gardner (2015). "Sire carcass breeding values affect body composition in lambs—1. Effects on lean weight and its distribution within the carcass as measured by computed tomography." Meat science **108**: 145-154.

Andrés-Bello, A., V. Barreto-Palacios, P. García-Segovia, J. Mir-Bel and J. Martínez-Monzó (2013). "Effect of pH on color and texture of food products." Food Engineering Reviews **5**(3): 158-170.

Anonymous (2005). "Handbook of Australian meat, International Red Meat Manual." **7th edition**.

Anonymous, J. A. R. (1992). "AUS-MEAT language, 4th edn." (AUS-MEAT: Sydney, Australia).

Antonini, E. (1965). "Interrelationship Between Structure and Function in Hemoglobin and Myoglobin." Physiological Reviews **4**: 123-170.

Arnold, R., S. Arp, K. Scheller, S. Williams and D. Schaefer (1993a). "Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef." Journal of animal science **71**(1): 105-118.

Arnold, R., K. Scheller, S. Arp, S. Williams and D. Schaefer (1993b). "Dietary α-Tocopheryl Acetate Enhances Beef Quality in Holstein and Beef Breed Steers." Journal of Food Science **58**(1): 28-33.

Arnold, R. N., K. K. Scheller, S. C. Arp, S. N. Williams, D. R. Buege and D. M. Schaefer (1992). "Effect of long- or short-term feeding of alpha-tocopheryl acetate to Holstein and crossbred beef steers on performance, carcass characteristics, and beef color stability." Journal of Animal Science **70**(10): 3055-3065.

Ashmore, C. R. and L. Doerr (1971). "Comparative aspects of muscle fiber types in different species." Experimental neurology **31**(3): 408-418.

Atkinson, J. and M. Follett (1973). "Biochemical studies on the discoloration of fresh meat." International Journal of Food Science & Technology **8**(1): 51-58.

Atkinson, J. L. and M. J. Follett (1973). "Biochemical studies on the discoloration of fresh meat." International Journal of Food Science & Technology **8**(1): 51-58.

Banks, R. (2002). "Enhancing the value of meat from wool sheep: Is there a need for specialisation?" Wool Technology and Sheep Breeding **50**(4).

Banks, R. G., A. J. Ball and S. R. Field (2002). Young sire programs in meat sheep breeds in Australia - a vehicle for rapid genetic improvement and continuous reserach and development. Proceedings of Seventh World Congress of Genetics Applied Livestock Production, Montipellier, France, CD-ROM Communication No. 23-15.

Bekhit, A. E. D. and C. Faustman (2005). "Metmyoglobin reducing activity." Meat Science **71**(3): 407-439.

Bekhit, A. E. D., G. H. Geesink, M. A. Ilian, J. D. Morton and R. Bickerstaffe (2003). "The effects of natural antioxidants on oxidative processes and metmyoglobin reducing activity in beef patties." Food Chemistry **81**(2): 175-187.

Bekhit, A. E. D., G. H. Geesink, J. D. Morton and R. Bickerstaffe (2001). "Metmyoglobin reducing activity and colour stability of ovine longissimus muscle." Meat Science **57**(4): 427-435.

Bellof, G., E. Most and J. Pallauf (2007). "Concentration of copper, iron, manganese and zinc in muscle, fat and bone tissue of lambs of the breed German Merino Landsheep in the course of the growing period and different feeding intensities." Journal of Animal Physiology and Animal Nutrition **91**(3-4): 100-108.

Bendall, J. and A. Taylor (1972). "Consumption of oxygen by the muscles of beef animals and related species. II. Consumption of oxygen by post-rigor muscle." Journal of the Science of Food and Agriculture **23**(6): 707-719.

Boakye, K. and G. S. Mittal (1996). "Changes in colour of beef m. longissimus dorsi muscle during ageing." Meat Science **42**(3): 347-354.

Borch, E., M.-L. Kant-Muermans and Y. Blixt (1996). "Bacterial spoilage of meat and cured meat products." International Journal of Food Microbiology **33**(1): 103-120.

Brandstetter, A. M., B. Picard and Y. Geay (1998). "Muscle fibre characteristics in four muscles of growing bulls: I. Postnatal differentiation." Livestock Production Science **53**(1): 15-23.

Brandstetter, A. M., B. Picard and Y. Geay (1998). "Muscle fibre characteristics in four muscles of growing male cattle: II. Effect of castration and feeding level." Livestock Production Science **53**(1): 25-36.

Briand, M. (1981). "Metabolic types of muscle in the sheep: I. Myosin ATPase, glycolytic, and mitochondrial enzyme activities. ." European Journal of applied physiology and occupational physiology **46**(4): 347-358.

Brooks, J. (1929). "*Post mortem* formation of methaemoglobin in red muscle. ." Biochemical Journal **23**: 1391-1400.

Brooks, J. (1935). "The Oxidation of Haemoglobin to Methaemoglobin by Oxygen. II.--The Relation between the Rate of Oxidation and the Partial Pressure of Oxygen." Proceedings of the Royal Society of London. Series B, Biological Sciences **118**(811): 560-577.

Brown, D., A. Huisman, A. Swan, H. Graser, R. Woolaston, A. Ball, K. Atkins and R. Banks (2007). Genetic evaluation for the Australian sheep industry. Proceedings of the Association for the Advancement of Animal Breeding and Genetics.

Brown, D. J., A. Ball, A. E. Huisman, A. A. Swan, K. D. Atkins, H. U. Graser, R. Banks, P. Swan and R. Woolaston (2006). Sheep Genetics Australia: a national genetic evaluation system for Australian sheep. Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, Minas Gerais, Brazil, Instituto Prociência.

Brown, W. D. and L. B. Mebine (1969). "Autoxidation of oxymyoglobins." Journal of Biological Chemistry **244**(24): 6696-6701.

Brunori, M. (2001). "Nitric oxide, cytochrome-*c* oxidase and myoglobin." Trends in Biochemical Sciences **26**(1): 21-23.

Bünger, L., E. A. Navajas, L. Stevenson, N. R. Lambe, C. A. Maltin, G. Simm, A. V. Fisher and K. C. Chang (2009). "Muscle fibre characteristics of two contrasting sheep breeds: Scottish Blackface and Texel." Meat Science **81**(2): 372-381.

Butler-Hogg, B., M. Francombe and E. Dransfield (1984). "Carcass and meat quality of ram and ewe lambs." Animal production **39**(01): 107-114.

Callejas-Cárdenas, A. R., I. Caro, C. Blanco, L. H. Villalobos-Delgado, N. Prieto, R. Bodas, F. J. Giráldez and J. Mateo (2014). "Effect of vacuum ageing on quality changes of lamb steaks from early fattening lambs during aerobic display." Meat Science **98**(4): 646-651.

Calnan, H., R. H. Jacob, D. W. Pethick and G. E. Gardner (2016). "Production factors influence fresh lamb longissimus colour more than muscle traits such as myoglobin concentration and pH." Meat Science **119**: 41-50.

Calnan, H. B., R. H. Jacob, D. W. Pethick and G. E. Gardner (2014). "Factors affecting the colour of lamb meat from the longissimus muscle during display: The influence of muscle weight and muscle oxidative capacity." Meat Science **96**(2, Part B): 1049-1057.

Cassens, R. G., W. G. Hoekstra, E. C. Faltin and E. J. Briskey (1967). Zinc content and subcellular distribution in red vs. white porcine skeletal muscle.

Channon, H., S. Baud and P. Walker (2005). "Modified atmosphere packaging improves retail display life of lamb cuts with variation between loin and knuckle." Animal Production Science **45**(5): 585-592.

Channon, H. A., J. Reynolds and S. Baud (2001). Identifying pathways to ensure acceptable eating quality of pork. Final Report. Canberra, Australian Pork Limited.

Chow, C., Y. Ochiai, S. Watabe and K. Hashimoto (1987). "Autoxidation of bluefin tuna myoglobin associated with freezing and thawing." Journal of Food Science **52**(3): 589-591.

Chu, Y., D. Huffman, G. Trout and W. Egbert (1987). "Color and color stability of frozen restructured beef steaks: Effect of sodium chloride, tripolyphosphate, nitrogen atmosphere, and processing procedures." Journal of Food Science **52**(4): 869-875.

CIE (1976). "Recommendations on uniform color spaces-color difference equations, Psychometric Color Terms. ." Supplement No. 2 to CIE Publication No. 15 (E-1.3.1.) 1978, 1971/(TC-1-3).

Clydesdale, F. M. and E. M. Ahmed (1978). "Colorimetry — methodology and applications." C R C Critical Reviews in Food Science and Nutrition **10**(3): 243-301.

Cornforth, D. P. and W. Egbert (1985). "Effect of rotenone and pH on the color of pre-rigor muscle." Journal of Food Science **50**(1): 34-35.

Curtis, K., M. Dolling, P. Cronjé and D. K. Maxwell (2006). The sheep industry-Australia in the global scene. Proceedings of the 2006 Australian Sheep Industry Cooperative Research Centre Conference, Orange, Australia, 22-23 February 2006., Australian Sheep Industry CRC.

Daley, C., A. Abbott, P. Doyle, G. Nader and S. Larson (2010). "A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef." Nutrition Journal **9**(1): 10.

Dean, R. and C. Ball (1960). "Analysis of the myoglobin fractions on the surfaces of beef cuts." Food Technology **14**(6): 271-286.

Dransfield, E., G. Nute, B. Hogg and B. Walters (1990). "Carcass and eating quality of ram, castrated ram and ewe lambs." Animal production **50**(02): 291-299.

Echevarne, C., M. Renerre and R. Labas (1990). "Metmyoglobin reductase activity in bovine muscles." Meat Science **27**(2): 161-172.

Eikelenboom, G., A. Hoving-Bolink, I. Kluitman, J. Houben and R. Klont (2000). "Effect of dietary vitamin E supplementation on beef colour stability." Meat Science **54**(1): 17-22.

England, E., T. Scheffler, S. Kasten, S. Matarneh and D. Gerrard (2013). "Exploring the unknowns involved in the transformation of muscle to meat." Meat science **95**(4): 837-843.

Essén-Gustavsson, B., A. Karlsson, K. Lundström and A. C. Enfält (1994). "Intramuscular fat and muscle fibre lipid contents in halothane-gene-free pigs fed high or low protein diets and its relation to meat quality." Meat Science **38**(2): 269-277.

Faustman, C. (2004). Food from supplement-fed animals. Technology of reduced-additive foods. Iowa, Blackwell 160-194.

Faustman, C. and R. G. Cassens (1990). "The biochemical basis for discoloration in fresh meat: A review." Journal of Muscle Foods **1**(3): 217-243.

Faustman, C., R. G. Cassens, D. M. Schaefer, D. R. Buege, S. N. Williams and K. K. Scheller (1989). "Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E." Journal of Food Science **54**(4): 858-862.

Faustman, C., W. K. M. Chan, D. M. Schaefer and A. Havens (1998). "Beef Color Update: The Role for Vitamin E." Journal of Animal Science **76**(4): 1019-1026.

Faustman, C., D. Liebler and J. Burr (1999). "α-Tocopherol oxidation in beef and in bovine muscle microsomes." Journal of agricultural and food chemistry **47**(4): 1396-1399.

Faustman, C., D. C. Liebler, T. D. McClure and Q. Sun (1999). "α, β-Unsaturated aldehydes accelerate oxymyoglobin oxidation." Journal of agricultural and food chemistry **47**(8): 3140-3144.

Faustman, C., Q. Sun, R. Mancini and S. P. Suman (2010). "Myoglobin and lipid oxidation interactions: Mechanistic bases and control." Meat Science **86**(1): 86-94.

Fiems, L. O., S. D. Campeneere, S. De Smet, G. Van de Voorde, J. M. Vanacker and C. V. Boucqué (2000). "Relationship between fat depots in carcasses of beef bulls and effect on meat colour and tenderness." Meat Science **56**(1): 41-47.

Fogarty, N. (1995). "Genetic parameters for live weight, fat and muscle measurements, wool production and reproduction in sheep: a review." Animal Breeding Abstracts (United Kingdom).

Fogarty, N. M., R. R. Banks, J. H. J. van der Werf, A. A. Ball and J. P. Gibson (2007). "The information nucleus- a new concept to enhance sheep industry genetic improvement. ." Proceedings of the Association for Advancement of Animal Breeding and Genetics **17**: 29-32.

Fogarty, N. M., A. R. Gilmour and D. L. Hopkins (1997). The relationship of crossbred lamb growth and carcass traits with lambplan EBVs of sires. Association for the Advancement of Animal Breeding and Genetics. , Dubbo, NSW, AAABG Distribution Service AGBU.

Forrest, J. C., E. D. Aberle, H. B. Hedrick, M. D. Judge and R. A. Merkel (1975). Principles of meat science, WH Freeman and Co.

Fox Jr, J. B. (1966). "Chemistry of meat pigments." Journal of Agricultural and Food Chemistry **14**(3): 207-210.

Fry, J. M., G. M. Smith, M. C. Mcgrath, E. J. Speijers and J. G. Allen (1993). "Plasma and tissue concentrations of α-tocopherol during vitamin E depletion in sheep." British Journal of Nutrition **69**(01): 225-232.

Gao, X., Z. Wang, J. Miao, L. Xie, Y. Dai, X. Li, Y. Chen, H. Luo and R. Dai (2014). "Influence of different production strategies on the stability of color, oxygen consumption and metmyoglobin reducing activity of meat from Ningxia Tan sheep." Meat Science **96**(2): 769-774.

Gardner, G. E., D. L. Hopkins, P. L. Greenwood, M. A. Cake, M. D. Boyce and D. W. Pethick (2007). "Sheep genotype, age and muscle type affect the expression of metabolic enzyme markers." Australian Journal of Experimental Agriculture **47**(10): 1180-1189.

Gardner, G. E., L. Kennedy, J. T. B. Milton and D. W. Pethick (1999). "Glycogen metabolism and ultimate pH of muscle in Merino, first-cross, and second-cross wether lambs as affected by stress before slaughter." Australian journal of agricultural research.

Gardner, G. E., D. W. Pethick, P. L. Greenwood and R. S. Hegarty (2006). "The effect of genotype and plane of nutrition on the rate of pH decline in lamb carcasses and the expression of metabolic enzymatic markers." Australian Journal of Agricultural Research **57**(6): 661-670.

Gardner, G. E., A. Williams, A. J. Ball, R. H. Jacob, G. Refshauge, J. Hocking Edwards, R. Behrendt and D. W. Pethick (2015). "Carcase weight and dressing percentage are increased using Australian Sheep Breeding Values for increased weight and muscling and reduced fat depth." Meat Science **99**: 89-98.

Gardner, G. E., A. Williams, J. Siddell, A. J. Ball, S. Mortimer, R. H. Jacob, K. L. Pearce, J. E. Hocking Edwards, J. B. Rowe and D. W. Pethick (2010). "Using Australian Sheep Breeding Values to increase lean meat yield percentage." Animal Production Science **50**(12): 1098-1106.

Garry, D. J. and P. P. Mammen (2007). Molecular insights into the functional role of myoglobin. Hypoxia and tHe circulation, Springer**:** 181-193.

Gašperlin, L., B. Žlender and V. Abram (2001). "Colour of beef heated to different temperatures as related to meat ageing." Meat Science **59**(1): 23-30.

Geesink, G., J. Robertson and A. Ball (2015). "The effect of retail packaging method on objective and consumer assessment of beef quality traits." Meat science **104**: 85-89.

George, A., J. Bendall and R. Jones (1980). "The tenderising effect of electrical stimulation of beef carcasses." Meat Science **4**(1): 51-68.

George, P. and C. Stratmann (1952). "The oxidation of myoglobin to metmyoglobin by oxygen. 2. The relation between the first order rate constant and the partial pressure of oxygen." Biochemical Journal **51**(3): 418.

Giddings, G. G. and H. O. Hultin (1974). "Reduction of ferrimyoglobin in meat." C R C Critical Reviews in Food Technology **5**(2): 143-173.

Giddings, G. G. and M. Solberg (1977). "The basis of color in muscle foods." C R C Critical Reviews in Food Science and Nutrition **9**(1): 81-114.

Govindarajan, S., H. O. Hultin and A. W. Kotula (1977). "Myoglobin Oxidation in Ground Beef: Mechanistic Studies." Journal of Food Science **42**(3): 571-577.

Greenwood, P. L., G. E. Gardner and R. S. Hegarty (2006). "Lamb myofibre characteristics are influenced by sire estimated breeding values and pastoral nutritional system." Australian Journal of Agricultural Research **57**(6): 627-639.

Greenwood, P. L., S. Harden and D. L. Hopkins (2007). "Myofibre characteristics of ovine longissimus and semitendinosus muscles are influenced by sire breed, gender, rearing type, age and carcass weight." Australian Journal of Experimental Agriculture **47**(10): 1137-1146.

Guidera, J., J. P. Kerry, D. J. Buckley, P. B. Lynch and P. A. Morrissey (1997). "The effect of dietary vitamin E supplementation on the quality of fresh and frozen lamb meat." Meat Science **45**(1): 33-43.

Gutzke, D. and G. R. Trout (2002). "Temperature and pH dependence of the autoxidation rate of bovine, ovine, porcine, and cervine oxymyoglobin isolated from three different muscles longissimus dorsi, gluteus medius, and biceps femoris." Journal of agricultural and food chemistry **50**(9): 2673-2678.

Hall, D., A. Gilmour, N. Fogarty and P. Holst (2002). "Growth and carcass composition of second-cross lambs. 2. Relationship between estimated breeding values of sires and their progeny performance under fast and slow growth regimes." Crop and Pasture Science **53**(12): 1341-1348.

Hall, D. G. (2000). "Opportunities for meat from lambs and goats in Australia." Asian-Australasian Journal of Animal Sciences **13**(SUPPL. 1): 91-101.

Hall, D. G. (2000). "Opportunities for meat from lambs and goats in Australia." Asian Australian Journal of Animal Sciences **13**: 91-92.

Hall, D. G., A. R. Gilmour, N. M. Fogarty and P. J. Holst (2002). "Growth and carcass composition of second-cross lambs. 2. Relationship between estimated breeding values of sires and their progeny and their performance under fast and slow growth regimes. ." Australian Journal of Agricultural Research **53**: 1341-1348.

Hall, D. G., A. R. Gilmour, N. M. Fogarty and P. J. Holst (2002). "Growth and carcass composition of second-cross lambs. 2. Relationship between estimated breeding values of sires and their progeny performance under fast and slow growth regimes." Australian Journal of Agricultural Research **53**(12): 1341.

Hall, D. G., A. R. Gilmour, N. M. Fogarty and P. J. Holst (2002). "Growth and carcass composition of second-cross lambs. 2. Relationship between estimated breeding values of sires and their progeny performance under fast and slow growth regimes." Australian Journal of Agricultural Research **53**(12): 1341-1348.

Hawkins, R. R., W. G. Moody and J. D. Kemp (1985). "Influence of genetic type, slaughter weight and sex on ovine muscle fiber and fat-cell development." Journal of Animal Science **61**: 1154-1163.

Hegarty, R. S., D. L. Hopkins, T. Farell, R. Banks and S. Harden (2006). "Effects of available nutrition on the growth and muscling potential of sires on the development of crossbred lambs: 2. Composition and commercial yield. ." Australian Journal of Agricultural Research **57**: 617-626.

Hegarty, R. S., D. L. Hopkins, T. C. Farrell, R. Banks and S. Harden (2006). "Effects of available nutrition and sire breeding values for growth and muscling on the development of crossbred lambs. 2: Composition and commercial yield." Australian Journal of Agricultural Research **57**(6): 617-626.

Hegarty, R. S., C. Shands, R. Marchant, D. L. Hopkins, A. J. Ball and S. Harden (2006). "Effects of available nutrition and sire breeding values for growth and muscling on the development of crossbred lambs. 1: Growth and carcass characteristics." Australian Journal of Agricultural Research **57**(6): 593-603.

Higgins, F., J. Kerry, D. Buckley and P. Morrissey (1998). "Dietary supplementation versus direct post mortem addition of α-tocopherol on lipid and colour stability in cooked turkey breast patties." Food research international **31**(3): 205-209.

Hocquette, J. F., F. Gondret, E. Baéza, F. Médale, C. Jurie and D. W. Pethick (2010). "Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers." Animal **4**(02): 303-319.

Hocquette, J. F., C. Jurie, Y. Ueda, P. Boulesteix, D. Bauchart and D. Pethick (2003). "The relationship between muscle metabolic pathways and marbling of beef." Progress in Research on Energy and Protein Metabolism **109**: 417-435.

Hocquette, J. F., I. Ortigues-Marty, D. Pethick, P. Herpin and X. Fernandez (1998). "Nutritional and hormonal regulation of energy metabolism in skeletal muscles of meat-producing animals." Livestock Production Science **56**(2): 115-143.

Holman, B. W., E. N. Ponnampalam, R. J. van de Ven, M. G. Kerr and D. L. Hopkins (2015). "Lamb meat colour values (HunterLab CIE and reflectance) are influenced by aperture size (5mm v. 25mm)." Meat science **100**: 202-208.

Hood, D. E. (1980). "Factors affecting the rate of metmyoglobin accumulation in pre-packaged beef." Meat Science **4**(4): 247-265.

Hood, D. E. and E. B. Riordan (1973). "Discolouration in pre-packaged beef: measurement by reflectance spectrophotometry and shopper discrimination." International Journal of Food Science & Technology **8**(3): 333-343.

Hopkins, D. (1996). "Assessment of lamb meat colour." Meat Focus International **5**(Part 11): 400-401.

Hopkins, D., M. Kerr, T. A. Lamb and R. Jacob (2008). Aperture size considerations when measuring colour stability of lamb cuts. Proceedings 54th international congress of meat science and technology.

Hopkins, D., T. Lamb, M. Kerr, R. van de Ven and E. Ponnampalam (2013). "Examination of the effect of ageing and temperature at rigor on colour stability of lamb meat." Meat science **95**(2): 311-316.

Hopkins, D., D. Stanley, E. Toohey, G. Gardner, D. Pethick and R. Van De Ven (2007). "Sire and growth path effects on sheep meat production 2. Meat and eating quality." Animal Production Science **47**(10): 1219-1228.

Hopkins, D. L. and N. M. Fogarty (1998). "Diverse lamb genotypes—2. Meat pH, colour and tenderness." Meat Science **49**(4): 477-488.

Hopkins, D. L., R. S. Hegarty and T. C. Farrell (2005). "Relationship between sire estimated breeding values and the meat and eating quality of meat from their progeny grown on two planes of nutrition." Australian Journal of Experimental Agriculture **45**(5): 525-533.

Hopkins, D. L., R. S. Hegarty, P. J. Walker and D. W. Pethick (2006). "Relationship between animal age, intramuscular fat, cooking loss, pH, shear force and eating quality of aged meat from sheep." Australian Journal of Experimental Agriculture **46**(7): 879-884.

Hopkins, D. L., D. F. Stanley, L. C. Martin, E. S. Toohey and A. R. Gilmour (2007). "Genotype and age effects on sheep meat production 3. Meat quality." Australian Journal of Experimental Agriculture **47**(10): 1155-1164.

Hoppeler, H. (1985). "Muscle aerobic potential in the animal kingdom." Proceedings of the Sixth International Symposium on the Biochemistry of exercise **6**: 417-435.

Horcada, A., M. Beriain, A. Purroy, G. Lizaso and J. Chasco (1998). "Effect of sex on meat quality of Spanish lamb breeds (Lacha and Rasa Aragonesa)." Animal Science **67**(03): 541-547.

Hughes, J. M., S. K. Oiseth, P. P. Purslow and R. D. Warner (2014). "A structural approach to understanding the interactions between colour, water-holding capacity and tenderness." Meat Science **98**(3): 520-532.

Huisman, A. E. and D. J. Brown (2008). "Genetic parameters for bodyweight, wool, and disease resistance and reproduction traits in Merino sheep. 2. Genetic relationships between bodyweight traits and other traits." Australian Journal of Experimental Agriculture **48**(9): 1186-1193.

Huisman, A. E. and D. J. Brown (2009). "Genetic parameters for bodyweight, wool, and disease resistance and reproduction traits in Merino sheep. 3. Genetic relationships between ultrasound scan traits and other traits." Animal Production Science **49**(4): 283-288.

Hunt, M., O. Sørheim and E. Slinde (1999). "Color and heat denaturation of myoglobin forms in ground beef." Journal of Food Science **64**(5): 847-851.

Hunt, M. C. (1980). Meat Colour Measurements. 33rd Annual Reciprocal Meat Conference of the American Meat Science Association Purdue University, West Lafayette, Indiana, National Live Stock and Meat Board.

Hunt, M. C. and H. B. Hedrick (1977). "Profile of fiber types and related properties of five bovine muscles." Journal of Food Science **42**(2): 513-517.

Hutchins, B. K., T. H. P. Liu and B. M. Watts (1967). "Effect of additives and refrigeration on reducing activity, metmyoglobin and malonaldehyde of raw ground beef." Journal of Food Science **32**(2): 214-217.

Ismail, H. A., E. J. Lee, K. Y. Ko and D. U. Ahn (2008). "Effects of aging time and natural antioxidants on the color, lipid oxidation and volatiles of irradiated ground beef." Meat Science **80**(3): 582-591.

Jacob, R. H., M. F. D'Antuono, A. R. Gilmour and R. D. Warner (2012). "Phenotypic characterization of colour stability of lamb meat." Meat Science(0).

Jacob, R. H., M. F. D'Antuono, A. R. Gilmour and R. D. Warner (2014). "Phenotypic characterisation of colour stability of lamb meat." Meat Science **96**(2, Part B): 1040-1048.

Jacob, R. H., M. F. D'Antuono, A. R. Gilmour and R. D. Warner (2014). "Phenotypic characterisation of colour stability of lamb meat." Meat science **96**(2): 1040-1048.

Jacob, R. H., M. F. D'Antuono, G. M. Smith, D. W. Pethick and R. D. Warner (2007). "Effect of lamb age and electrical stimulation on the colour stability of fresh lamb meat." Crop and Pasture Science **58**(4): 374-382.

Jakobsen, M. and G. Bertelsen (2000). "Colour stability and lipid oxidation of fresh beef. Development of a response surface model for predicting the effects of temperature, storage time, and modified atmosphere composition." Meat Science **54**(1): 49-57.

Jensen, M., A. Lindholm and J. Hakkarainen (1989). "The vitamin E distribution in serum, liver, adipose and muscle tissues in the pig during depletion and repletion." Acta Veterinaria Scandinavica **31**(2): 129-136.

Jeyamkondan, S., D. S. Jayas and R. A. Holley (2000). "Review of centralized packaging systems for distribution of retail-ready meat." Journal of Food Protection® **63**(6): 796-804.

Jeyamkondan, S., D. S. Jayas and R. A. Holley (2000). "Review of centralized packaging systems for distribution of retail-ready meet." Journal of Food Protection **63**(6): 796-804.

Jose, C. (2011). "Anti- and Pro-oxidant interactions with myoglobin in Lamb meat, and the influence on colour stability." PhD thesis, Murdoch University.

Jose, C., P. McGilchrist, J. Perovic, G. E. Gardner and D. Pethick (2015). "The economic impact of dark cutting beef in Australia." 61st International Congress of Meat Science and Technology.

Jose, C., D. Pethick, G. Gardner and R. Jacob (2008). The colour stability of aged lamb benefits from Vitamin E supplementation. Proceedings 54th international congress of meat science and technology.

Jose, C., D. Pethick, G. Gardner and R. Jacob (2008). Vitamin E will improve the colour stability in lamb; a dose rate investigation. 54th international congress of meat science and technology.

Jose, C. G., R. H. Jacob, D. W. Pethick and G. E. Gardner (2016). "Short term supplementation rates to optimise vitamin E concentration for retail colour stability of Australian lamb meat." Meat Science **111**: 101-109.

Jurie, C., J.-F. Martin, A. Listrat, R. Jailler, J. Culioli and B. Picard (2005). "Effects of age and breed of beef bulls on growth parameters, carcass and muscle characteristics." Animal Science **80**(03): 257-263.

Kelman, K., L. Pannier, G. E. Gardner and D. Pethick (2012). "The impact of selection for lean meat yield in lambs on muscle oxidative metabolism." Meat Science (CRC Special Issue).

Kelman, K. R., L. Pannier, D. W. Pethick and G. E. Gardner (2014). "Selection for lean meat yield in lambs reduces indicators of oxidative metabolism in the longissimus muscle." Meat Science **96**(2, Part B): 1058-1067.

Khliji, S., R. Van de Ven, T. A. Lamb, M. Lanza and D. L. Hopkins (2010). "Relationship between consumer ranking of lamb colour and objective measures of colour." Meat Science **85**(2): 224-229.

Kim, Y. and M. Hunt (2011). "Advance technology to improve meat color. In 'Control of meat quality.'(Ed. ST Joo) pp. 31–60." Research Signpost: Trivandrum, Kerala, India.

Kim, Y. H. B., M. Frandsen and K. Rosenvold (2011). "Effect of ageing prior to freezing on colour stability of ovine longissimus muscle." Meat science **88**(3): 332-337.

Kim, Y. H. B., G. Luc and K. Rosenvold (2013). "Pre rigor processing, ageing and freezing on tenderness and colour stability of lamb loins." Meat science **95**(2): 412-418.

Kim, Y. H. B., A. Stuart, C. Black and K. Rosenvold (2012). "Effect of lamb age and retail packaging types on the quality of long-term chilled lamb loins." Meat science **90**(4): 962-966.

King, D. A., S. D. Shackelford, N. Kalchayanand and T. L. Wheeler (2012). "Sampling and aging effects on beef longissimus color stability measurements." Journal of animal science **90**(10): 3596.

King, D. A., S. D. Shackelford, L. A. Kuehn, C. M. Kemp, A. B. Rodriguez, R. M. Thallman and T. L. Wheeler (2010). "Contribution of genetic influences to animal-to-animal variation in myoglobin content and beef lean color stability." Journal of Animal Science **88**(3): 1160-1167.

Klont, R. E., L. Brocks and G. Eikelenboom (1998). "Muscle fibre type and meat quality." Meat Science **49**: S219-S229.

Klont, R. E., Brocks, L., Eikelenboom, G. (1998). "Muscle fibre type and meat quality." Meat Science **49**: S219-S229.

Kondo, H., M. Kimura and Y. Itokawa (1991). "Manganese, Copper, Zinc, and Iron Concentrations and Subcellular Distribution in Two Types of Skeletal Muscle." Experimental Biology and Medicine **196**(1): 83-88.

Kropf, D. (1993). "Colour stability. Factors affecting the colour of fresh meat." Meat Focus International (United Kingdom).

Lanari, M. C. and R. G. Cassens (1991). "Mitochondrial Activity and Beef Muscle Color Stability." Journal of Food Science **56**(6): 1476-1479.

Lanari, M. C., R. G. Cassens, D. M. Schaefer and K. K. Scheller (1993). "Dietary Vitamin E Enhances Color and Display Life of Frozen Beef from Holstein Steers." Journal of Food Science **58**(4): 701-704.

Lanari, M. C., R. G. Cassens, D. M. Schaefer and K. K. Scheller (1994). "Effect of dietary vitamin E on pigment and lipid stability of frozen beef: A kinetic analysis." Meat Science **38**(1): 3-15.

Lauzurica, S., J. de la Fuente, M. T. Díaz, I. Álvarez, C. Pérez and V. Cañeque (2005). "Effect of dietary supplementation of vitamin E on characteristics of lamb meat packed under modified atmosphere." Meat Science **70**(4): 639-646.

Lawrie, R. A. (1958). "Physiological stress in relation to dark-cutting beef." Journal of Science Food and Agriculture **9**: 721.

Lawrie, R. A. (1983). "Aspects of the biochemistry of meat." International Journal of Biochemistry **15**(3): 233-242.

Ledward, D. (1972). "Metmyoglobin reduction and formation in beef during aerobic storage at 1° C " Journal of Food Science **37**(4): 634-635.

Ledward, D. A. (1970). "Metmyoglobin formation in beef stored in carbon dioxide enriched and oxygen depleted atmospheres." Journal of Food Science **35**(1): 33-37.

Ledward, D. A. (1985). "Post-slaughter influences on the formation of metyyoglobin in beef muscles." Meat Science **15**(3): 149-171.

Ledward, D. A. (1992). Haemoproteins in Meat and Meat Products. Biochemistry of Food Proteins. B. J. F. Hudson, Springer US**:** 197-234.

Ledward, D. A., R. F. Dickinson, V. H. Powell and W. R. Shorthose (1986). "The colour and colour stability of beef Longissimus dorsi and Semimembranosus muscles after effective electrical stimulation." Meat Science **16**(4): 245-265.

Ledward, D. A. and W. R. Shorthose (1971). "A note on the haem pigment concentration of lamb as influenced by age and sex." Animal Science **13**(01): 193-195.

Lee, M., J. Apple, J. Yancey, J. Sawyer and Z. Johnson (2008). "Influence of wet-aging on bloom development in the longissimus thoracis." Meat science **80**(3): 703-707.

Lee, S. H., S. T. Joo and Y. C. Ryu (2010). "Skeletal muscle fiber type and myofibrillar proteins in relation to meat quality." Meat Science **86**(1): 166-170.

Leedle, R. A. and S. D. Aust (1986). "Importance of the polyunsaturated fatty acid to vitamin E ratio in the resistance of rat lung microsomes to lipid peroxidation." Journal of free radicals in biology & medicine **2**(5-6): 397-403.

Lefaucheur, L. (2010). "A second look into fibre typing–Relation to meat quality." Meat Science **84**(2): 257-270.

Lefaucheur, L. (2010). "A second look into fibre typing – Relation to meat quality." Meat Science **84**(2): 257-270.

Li, Y. and S. Liu (2012). "Reducing lipid peroxidation for improving colour stability of beef and lamb: on-farm considerations." Journal of the Science of Food and Agriculture **92**(4): 719-729.

Lin, K. C., H. R. Cross, H. K. Johnson, B. C. Breidenstein, V. Randecker and R. A. Field (1988). "Mineral composition of lamb carcasses from the United States and New Zealand." Meat Science **24**(1): 47-59.

Lindahl, G., P. Henckel, A. H. Karlsson and H. J. Andersen (2006). "Significance of early postmortem temperature and pH decline on colour characteristics of pork loin from different crossbreeds." Meat science **72**(4): 613-623.

Linder, M. C. (1991). "Nutritional biochemistry and metabolism: with clinical applications."

Liu, Q., M. Lanari and D. Schaefer (1995). "A review of dietary vitamin E supplementation for improvement of beef quality." Journal of animal science **73**(10): 3131-3140.

Lorentzen, T. K. and O. Vangen (2012). "Genetic and phenotypic analysis of meat quality traits in lamb and correlations to carcass composition." Livestock Science **143**(2–3): 201-209.

Luciano, G., F. J. Monahan, V. Vasta, P. Pennisi, M. Bella and A. Priolo (2009). "Lipid and colour stability of meat from lambs fed fresh herbage or concentrate." Meat Science **82**(2): 193-199.

Lynch, M. P., J. P. Kerry, D. J. Buckley, C. Faustman and P. A. Morrissey (1999). "Effect of dietary vitamin E supplementation on the colour and lipid stability of fresh, frozen and vacuum-packaged beef." Meat Science **52**(1): 95-99.

MacDougall, D., I. Bremner and A. Dalgarno (1973). "Effect of dietary iron on the colour and pigment concentration of veal." Journal of the Science of Food and Agriculture **24**(10): 1255-1263.

MacDougall, D. B. (1982). "Changes in the colour and opacity of meat." Food Chemistry **9**(1): 75-88.

Machlin, L. J. (1984). Vitamin E. L. Machlin (Ed.) Handbook of Vitamins. New York, Marcel Dekker**:** 99.

Madhavi, D. L. and C. E. Carpenter (1993). "Aging and Processing Affect Color, Metmyoglobin Reductase and Oxygen Consumption of Beef Muscles." Journal of Food Science **58**(5): 939-942.

Mancini, R. A. and M. C. Hunt (2005). "Current research in meat color." Meat Science **71**(1): 100-121.

Mancini, R. A. and R. Ramanathan (2014). "Effects of postmortem storage time on color and mitochondria in beef." Meat Science **98**(1): 65-70.

Martin, K., P. McGilchrist, J. Thompson and G. Gardner (2011). "Progeny of high muscling sires have reduced muscle response to adrenaline in sheep." animal **5**(07): 1060-1070.

Martínez-Cerezo, S., C. Sañudo, B. Panea, I. Medel, R. Delfa, I. Sierra, J. A. Beltrán, R. Cepero and J. L. Olleta (2005). "Breed, slaughter weight and ageing time effects on physico-chemical characteristics of lamb meat." Meat Science **69**(2): 325-333.

McDowell, L. R. (1989). Vitamins in Animal Nutrition; Comparative Aspects to Human Nutrition. San Diego, Academic Press Inc.

McGuire, R. G. (1992). "Reporting of objective color measurements." HortScience **27**(12): 1254-1255.

McKenna, D. R., P. D. Mies, B. E. Baird, K. D. Pfeiffer, J. W. Ellebracht and J. W. Savell (2005). "Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles." Meat Science **70**(4): 665-682.

McMillin, K. W. (2008). "Where is MAP Going? A review and future potential of modified atmosphere packaging for meat." Meat Science **80**(1): 43-65.

McMurray, C., W. Blanchflower and D. Rice (1980). "Influence of extraction techniques on determination of alpha-tocopherol in animal feedstuffs." Journal-Association of Official Analytical Chemists **63**(6): 1258-1261.

McPhee, M. J., D. L. Hopkins and D. W. Pethick (2008). "Intramuscular fat levels in sheep muscle during growth." Australian Journal of Experimental Agriculture **48**(7): 904-909.

Millikan, G. A. (1939). "Muscle Hemoglobin." Physiological Reviews **19**(4): 503-523.

Miltenburg, G., T. Wensing, F. Smulders and H. Breukink (1992). "Relationship between blood hemoglobin, plasma and tissue iron, muscle heme pigment, and carcass color of veal." Journal of Animal Science **70**(9): 2766-2772.

MLA (2005). Australian lamb product guide, Meat and Livestock Australia.

MLA (2016). MLA Fast Facts: Australia's sheep industry, Meat & Livestock Australia Limited.

Monin, G. and A. Ouali (1992). Muscle differentiation and meat quality. London, Elsevier Applied Science.

Moody, W. G., J. D. Kemp, M. Mahyuddin, D. M. Johnston and D. G. Ely (1980). "Effect of feeding systems, slaughter eight and sex on histological properties of lamb carcasses." Journal of Animal Science **20**(2): 249-256.

Moore, V. (1990). "Factors influencing frozen display life of lamb chops and steaks: effect of packaging and temperature." Meat science **27**(2): 91-98.

Moore, V. and C. Gill (1987). "The pH and display life of chilled lamb after prolonged storage under vacuum or under CO2." New Zealand Journal of Agricultural Research **30**(4): 449-452.

Moore, V. and O. Young (1991). "The effects of electrical stimulation, thawing, ageing and packaging on the colour and display life of lamb chops." Meat Science **30**(2): 131-145.

Morrissey, E., Jacob, R.H., Pluske J. (2008). "Perception of red brown colour by consumers." International Congress of Meat Science and Technology.

Mortimer, S. I., J. H. J. van der Werf, R. H. Jacob, D. L. Hopkins, L. Pannier, K. L. Pearce, G. E. Gardner, R. D. Warner, G. H. Geesink, J. E. Hocking Edwards, E. N. Ponnampalam, A. J. Ball, A. R. Gilmour and D. W. Pethick (2014). "Genetic parameters for meat quality traits of Australian lamb meat." Meat Science **96**(2, Part B): 1016-1024.

Mortimer, S. I., J. H. J. van der Werf, R. H. Jacob, D. W. Pethick, K. L. Pearce, R. D. Warner, G. H. Geesink, J. E. Hocking Edwards, E. N. Ponnampalam, B. A.J. and H. D.L. (2012). "Genetic parameters for meat quality traits of Australian lamb meat " Meat Science (CRC Special Issue).

Murray, R. K., D. K. Granner, P. A. Mayes and V. W. Rodwell (2004). Harper's Biochemistry. 2000. New York, McGrow-Hill.

Nelson, D. and M. Cox (2005). Lehninger principles of biochemistry (4th ed.). New York.

NRC (1985). Nutrient Requirements of Sheep, National Research Council, Subcommittee on Sheep Nutrition, Comittee on Animal Nutrition, Board on Agriculture, National Academy Press, Washington, D.C.

O'Grady, M., F. Monahan and N. Brunton (2001). "Oxymyoglobin oxidation and lipid oxidation in bovine muscle—mechanistic studies." Journal of Food Science **66**(3): 386-392.

O'Keeffe, M. and D. E. Hood (1982). "Biochemical factors influencing metmyoglobin formation on beef from muscles of differing colour stability." Meat Science **7**(3): 209-228.

Ogrydziak, D. and W. Brown (1982). "Temperature effects in modified-atmosphere storage of seafoods." Food Technology.

Ono, K., B. W. Berry, H. K. Johnson, E. Russek, C. F. Parker, V. R. Cahill and P. G. Althouse (1984). "Nutrient composition of lamb of two age groups." Journal of Food Science **49**(5): 1233-1239.

Ordway, G. A. and D. J. Garry (2004). "Myoglobin: an essential hemoprotein in striated muscle." Journal of Experimental Biology **207**(20): 3441-3446.

Pannier, L., D. Pethick, G. H. Geesink, A. J. Ball, R. Jacob and G. E. Gardner (2012). "Intramuscular fat is reduced in lambs from sires selected for leanness." Meat Science (CRC Special Issue).

Pannier, L., D. W. Pethick, M. D. Boyce, A. J. Ball, R. H. Jacob and G. E. Gardner (2014). "Associations of genetic and non-genetic factors with concentrations of iron and zinc in the longissimus muscle of lamb." Meat science **96**(2): 1111-1119.

Pannier, L., D. W. Pethick, M. D. Boyce, A. J. Ball, R. H. Jacob and G. E. Gardner (2014). "Associations of genetic and non-genetic factors with concentrations of iron and zinc in the longissimus muscle of lamb." Meat Science **96**(2, Part B): 1111-1119.

Pannier, L., D. W. Pethick, G. H. Geesink, A. J. Ball, R. H. Jacob and G. E. Gardner (2014). "Intramuscular fat in the longissimus muscle is reduced in lambs from sires selected for leanness." Meat Science **96**(2, Part B): 1068-1075.

Pannier, L., E. N. Ponnampalam, G. E. Gardner, D. L. Hopkins, A. J. Ball, R. H. Jacob, K. L. Pearce and D. W. Pethick (2010). "Prime Australian lamb supplies key nutrients for human health." Animal Production Science **50**(12): 1115-1122.

Papas, A. (1999). "Diet and antioxidant status." Food and Chemical Toxicology **37**(9): 999-1007.

Pearce, K. L., D. G. Masters, G. M. Smith, R. H. Jacob and D. W. Pethick (2005). "Plasma and tissue α-tocopherol concentrations and meat colour stability in sheep

grazing saltbush (Atriplex spp.)." Australian Journal of Agricultural Research **56**(7): 663-672.

Pearce, K. L., L. Pannier, A. Williams, G. E. Gardner, A. Ball and D. W. Pethick (2009). "Factors affecting the iron and zinc content of lamb." Recent advances in animal nutrition–Australia **17**: 37-42.

Pearce, K. L., R. van de Ven, C. Mudford, R. D. Warner, J. Hocking-Edwards, R. Jacob, D. W. Pethick and D. L. Hopkins (2010). "Case studies demonstrating the benefits on pH and temperature decline of optimising medium-voltage electrical stimulation of lamb carcasses." Animal Production Science **50**(12): 1107-1114.

Pearson, A., J. D. Love and F. Shorland (1977). "'"Warmed-over" flavor in meat, poultry, and fish." Advances in food research **23**: 1-74.

Penney, N. and R. Bell (1993). "Effect of residual oxygen on the colour, odour and taste of carbon dioxide-packaged beef, lamb and pork during short term storage at chill temperatures." Meat science **33**(2): 245-252.

Perry, D., Shorthose, W.R., Ferguson, D.M., & Thompson, J.M. (2001). "Methods used in the CRC program for the determination of carcass yeild and beef quality." Australian Journal of Experimental Agriculture **71**(7): 953-957.

Peter, J. B., R. J. Barnard, V. R. Edgerton, C. A. Gillespie and K. E. Stempel (1972). "Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits." Biochemistry **11**(14): 2627-2633.

Pethick, D. W., R. G. Banks, J. Hales and I. R. Ross (2006). "Australian prime lamb–a vision for 2020." International Journal of Sheep and Wool Science **54**(1).

Pethick, D. W., Banks, R.G., Hales, J. and Ross,I.R. (2006). "Australian prime lamb- a vision for 2020." International Journal of Sheep and Wool Science **54**: 66-73.

Pethick, D. W., D. L. Hopkins, D. N. D'Souza, J. M. Thompson and P. J. Walker (2005). "Effects of animal age on the eating quality of sheep meat." Australian Journal of Experimental Agriculture **45**(5): 491-498.

Petron, M., K. Raes, E. Claeys, M. Lourenço, D. Fremaut and S. De Smet (2007). "Effect of grazing pastures of different botanical composition on antioxidant enzyme activities and oxidative stability of lamb meat." Meat science **75**(4): 737-745.

Picard, B., L. Lefaucheur, C. Berri and M. J. Duclos (2002). "Muscle fibre ontogenesis in farm animal species." Reproduction Nutrition Development **42**(5): 415-432.

Polkinghorne, R., R. Watson, M. Porter, A. Gee, J. Scott and J. Thompson (1999). Meat standards Australia, APACCP'based beef grading scheme for consumers. 1) The use of consumer scores to set grade standards. International congress of meat science and technology

Ponnampalam, E. N., K. L. Butler, V. F. Burnett, M. B. McDonagh, J. L. Jacobs and D. L. Hopkins (2013). "Aged vacuum packaged lamb cuts are less brown than fresh muscle cuts under simulated retail display."

Ponnampalam, E. N., K. L. Butler, R. H. Jacob, D. W. Pethick, A. J. Ball, J. E. Hocking Edwards, G. Geesink and D. L. Hopkins (2014). "Health beneficial long chain omega-3 fatty acid levels in Australian lamb managed under extensive finishing systems." Meat Science **96**(2, Part B): 1104-1110.

Ponnampalam, E. N., K. L. Butler, R. H. Jacob, D. W. Pethick, A. J. Ball, J. E. Hocking Edwards, G. H. Geesink and D. L. Hopkins (2012). "Health beneficial long chain omega-3 fatty acids in Australian lambs managed under extensive finishing systems." Meat Science (Submitted).

Ponnampalam, E. N., K. L. Butler, M. B. McDonagh, J. L. Jacobs and D. L. Hopkins (2012). "Relationship between muscle antioxidant status, forms of iron, polyunsaturated fatty acids and functionality (retail colour) of meat in lambs." Meat Science **90**(2): 297-303.

Ponnampalam, E. N., G. R. Trout, A. J. Sinclair, A. R. Egan and B. J. Leury (2001). "Comparison of the color stability and lipid oxidative stability of fresh and vacuum packaged lamb muscle containing elevated omega-3 and omega-6 fatty acid levels from dietary manipulation." Meat Science **58**(2): 151-161.

Porter, K. R. and G. E. Palade (1957). "Studies on the endoplasmic reticulum: III. Its form and distribution in striated muscle cells." The Journal of biophysical and biochemical cytology **3**(2): 269.

Priolo, A., D. Micol, J. Agabriel, S. Prache and E. Dransfield (2002). "Effect of grass or concentrate feeding systems on lamb carcass and meat quality." Meat Science **62**(2): 179-185.

Ramanathan, R., M. Konda, R. Mancini and C. Faustman (2009). "Species-Specific Effects of Sarcoplasmic Extracts on Lipid Oxidation in vitro." Journal of food science **74**(1): C73-C77.

Ramírez-Retamal, J. and R. Morales (2014). "Influence of breed and feeding on the main quality characteristics of sheep carcass and meat: A review." Chilean Journal of Agricultural Research **74**(2): 225-233.

Ray, B. (1996). Control of modified atmosphere (or reducing O-R potential). Fundamental food microbiology. Florida, CRC Press**:** 417-422.

Realini, C., S. Duckett, G. Brito, M. Dalla Rizza and D. De Mattos (2004). "Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef." Meat Science **66**(3): 567-577.

Reddy, L. M. and C. E. Carpenter (1991). "Determination of Metmyoglobin Reductase Activity in Bovine Skeletal Muscles." Journal of Food Science **56**(5): 1161-1164.

Renerre, M. (1990). "Factors involved in the discoloration of beef meat." International Journal of Food Science & Technology **25**(6): 613-630.

Renerre, M. (2000). Oxidative processes and myoglobin. Antioxidants in muscle foods, John Wiley & Sons, Inc**:** 113-133.

Renerre, M. and J. Labadie (1993). Fresh meat packaging and meat quality. Proceedings of the 39th International Congress of Meat Science and Technology, Calgary, Canada.

Renerre, M. and R. Labas (1987). "Biochemical factors influencing metmyoglobin formation in beef muscles." Meat Science **19**(2): 151-165.

Rikert, J., L. Bressler, C. Ball and E. Steir (1957). "Factors affectingquality of prepackaged meat. II Colour studies. B. Effects of storage time, storage temperature, antioxidants, bacteria, light, freezing and fat upon colour of product. ." Food Technology **11**: 567-571.

Ripoll, G., M. Joy, F. Muñoz and P. Albertí (2008). "Meat and fat colour as a tool to trace grass-feeding systems in light lamb production." Meat Science **80**(2): 239-248.

Robbins, K., J. Jensen, K. Ryan, C. Homco-Ryan, F. McKeith and M. Brewer (2003). "Dietary vitamin E supplementation effects on the color and sensory characteristics of enhanced beef steaks." Meat science **64**(3): 279-285.

Rosenvold, K. and E. Wiklund (2011). "Retail colour display life of chilled lamb as affected by processing conditions and storage temperature." Meat Science **88**(3): 354-360.

Salviati, G., R. Betto, A. Margreth, F. Novello and E. Bonetti (1980). "Differential binding of vitamin E to sarcoplasmic reticulum from fast and slow muscles of the rabbit." Cellular and Molecular Life Sciences **36**(10): 1140-1141.

Sammel, L. M., M. C. Hunt, D. H. Kropf, K. A. Hachmeister and D. E. Johnson (2002). "Comparison of assays for metmyoglobin reducing ability in beef inside and outside semimembranosus muscle." Journal of Food Science **67**(3): 978-984.

Savell, J., G. Smith and Z. Carpenter (1978). "Beef quality and palatability as affected by electrical stimulation and cooler aging." Journal of Food Science **43**(6): 1666-1668.

Savell, J. W. and H. R. Cross (1988). The role of fat in the palatability of beef, pork, and lamb. Designing foods: Animal product options in the marketplace/Committee on technological options to improve the nutritional attributes of animal products, Board of Agriculture, National Research Council**:** 345.

Schiaffino, S. and C. Reggiani (1996). "Molecular diversity of myofibrillar proteins: gene regulation and functional significance." Physiological reviews **76**(2): 371-423.

Schreurs, N. M., F. Garcia, C. Jurie, J. Agabriel, D. Micol, D. Bauchart, A. Listrat and B. Picard (2008). "Meta-analysis of the effect of animal maturity on muscle characteristics in different muscles, breeds, and sexes of cattle." Journal of Animal Science **86**(11): 2872-2887.

Shikama, K. and Y. Sugawara (1978). "Autoxidation of native oxymyoglobin. Kinetic analysis of the pH profile." European Journal of Biochemistry **91**(2): 407-413.

Silliker, J., R. Woodruff, J. Lugg, S. Wolfe and W. Brown (1977). "Preservation of refrigerated meats with controlled atmospheres: treatment and post-treatment effects of carbon dioxide on pork and beef." Meat Science **1**(3): 195-204.

Sleper, P., M. Hunt, D. Kropf, C. Kastner and M. Dikeman (1983). "Electrical stimulation effects on myoglobin properties of bovine longissimus muscle." Journal of Food Science **48**(2): 479-483.

Smith, G., J. Morgan, J. Sofos and J. Tatum (1996). "Supplemental vitamin E in beef cattle diets to improve shelf-life of beef." Animal feed science and technology **59**(1-3): 207-214.

Smith, G. C., Belk, K.E., Sofos, J.N., Tatum, J.D. and Williams, S.N., (2000). Antioxidants in muscle foods: Nutritional strategies to improve quality. Wiley, New York, NY, pp.397-426. New York, NY, Wiley.

Sookhareea, R., D. Taylor, K. Woodford, G. Dryden and R. Shorthose (1995). The effect of slaughter age and sex type on meat quality of Javan rusa (Cervus timorensis) male deer. CSIRO Meat Industry Research Conference, CSIRO Publishing Australia.

Staron, R. S. and P. Johnson (1993). "Myosin polymorphism and differential expression in adult human skeletal muscle." Comparative Biochemistry and Physiology Part B: Comparative Biochemistry **106**(3): 463-475.

Strohecker, M. G., C. Faustman, H. Furr, T. A. Hoagland and S. N. Williams (1997). "Vitamin E supplementation effects on color and lipid stability of whole and ground lamb." Journal of Muscle Foods **8**(4): 413-426.

Stubbs, R., J. Morgan, F. Ray and H. Dolezal (2002). "Effect of supplemental vitamin E on the color and case-life of top loin steaks and ground chuck patties in modified atmosphere case-ready retail packaging systems." Meat science **61**(1): 1-5.

Suman, S. P., M. C. Hunt, M. N. Nair and G. Rentfrow (2014). "Improving beef color stability: Practical strategies and underlying mechanisms." Meat science **98**(3): 490-504.

Suman, S. P. and P. Joseph (2013). "Myoglobin Chemistry and Meat Color." Annual Review of Food Science and Technology **4**(1): 79-99.

Suzuki, A. and R. G. Cassens (1983). "A histochemical study of myofiber types in the serratus ventralis thoracis muscle of sheep during growth." Journal of Animal Science **56**(6): 1447-1458.

Swatland, H. (1995). "Reversible pH effect on pork paleness in a model system." Journal of food science **60**(5): 988-991.

Swatland, H. J. (2003). "Ellipsometry across isolated muscle fibres indicates a refractive contribution to paleness in pork." Meat Science **63**(4): 463-467.

Swatland, H. J. (2004). "Absorbance of light by mitochondira in pork muscle fibres differing in myoglobin content." Meat Science **67**: 371-375.

Swatland, H. J. (2012). Optical properties of meat. Proceedings of the 65th Reciprocal Meat Conference.

Takano, T. (1977). "Structure of myoglobin refined at 2· 0 Å resolution: I. Crystallographic refinement of metmyoglobin from sperm whale." Journal of molecular biology **110**(3): 537-568.

Tang, J., C. Faustman, T. A. Hoagland, R. A. Mancini, M. Seyfert and M. C. Hunt (2005). "Postmortem oxygen consumption by mitochondria and its effects on myoglobin form and stability." Journal of Agricultural and Food Chemistry **53**(4): 1223-1230.

Tapp, W., J. Yancey and J. Apple (2011). "How is the instrumental color of meat measured?" Meat Science **89**(1): 1-5.

Teixeira, A., S. Batista, R. Delfa and V. Cadavez (2005). "Lamb meat quality of two breeds with protected origin designation. Influence of breed, sex and live weight." Meat Science **71**(3): 530-536.

Tejeda, J. F., R. E. Peña and A. I. Andrés (2008). "Effect of live weight and sex on physico-chemical and sensorial characteristics of Merino lamb meat." Meat Science **80**(4): 1061-1067.

Thompson, J. (2004). "The effects of marbling on flavour and juiciness scores of cooked beef, after adjusting to a constant tenderness." Animal Production Science **44**(7): 645-652.

Thompson, J. M., J. R. Parks and D. Perry (1985). "Food intake, growth and body composition in Australian Merino sheep selected for high and low weaning weight. 1. Food intake, food efficiency and growth." Animal Science **40**(01): 55-70.

Toohey, E. and D. Hopkins (2006). "The impact of new generation pre-dressing mid-voltage electrical stimulation on colour stability." Meat Science and Technology.

Trout, G. R. (1991). "A rapid method for measuring pigment concentration in porcine and other low pigmented muscles." Proceedings of the 37th international congress of meat science and technology: 1198-1201.

Underwood, E. (2012). Trace Elements in Human and Animal Nutrition 4e, Elsevier.

Unruh, J., C. Kastner, D. Kropf, M. Dikeman and M. Hunt (1986). "Effects of low-voltage electrical stimulation during exsanguination on meat quality and display colour stability." Meat Science **18**(4): 281-293.

USEPA (1991). "Methods for the determination of metals in environmental samples." US Environmental Protection Agency EPA/600/604-691/010.

van de Werf, J. H. J., B. P. Kinghorn and R. G. Banks (2010). "Design and role of an information nucleus in sheep breeding programs." Animal Production Science **50**(11-12): 998-1003.

Vitale, M., M. Pérez-Juan, E. Lloret, J. Arnau and C. E. Realini (2014). "Effect of aging time in vacuum on tenderness, and color and lipid stability of beef from mature cows during display in high oxygen atmosphere package." Meat Science **96**(1): 270-277.

Walsh, H. M. and J. P. Kerry (2002). Meat packaging. Meat Processing. J. Kerry, J. Kerry and D. Ledward, Woodhead Publishing**:** 417-451.

Warner, R., G. Kearney, D. Hopkins and R. Jacob (2017). "Retail colour stability of lamb meat is influenced by breed type, muscle, packaging and iron concentration." Meat Science **129**: 28-37.

Warner, R. D., D. W. Pethick, P. L. Greenwood, E. N. Ponnampalam, R. G. Banks and D. L. Hopkins (2007). "Unravelling the complex interactions between genetics, animal age and nutrition as they impact on tissue deposition, muscle characteristics and quality of Australian sheep meat." Australian Journal of Experimental Agriculture **47**(10): 1229-1238.

Warner, R. D., E. N. Ponnampalam, G. A. Kearney, D. L. Hopkins and R. H. Jacob (2007). "Genotype and age at slaughter influence the retail shelf-life of the loin and knuckle from sheep carcasses." Australian Journal of Experimental Agriculture **47**(10): 1190-1200.

Warriss, P. D. (2000). Meat science: An introductory text. . Wallingford, CAB-International.

Wegner, J., E. Albrecht, I. Fiedler, F. Teuscher, H.-J. Papstein and K. Ender (2000). "Growth- and breed-related changes of muscle fiber characteristics in cattle." Journal of Animal Science **78**(6): 1485-1496.

Wegner, J., Albrecht, E., Fiedler, I., Teuscher, F., Papstein, H.J. and Ender, K. (2000). "Growth- and breed-related changes of muscle fiber characteristics in cattle." Journal of Animal Science **78**: 1485-1496.

White, N. A., M. D. McGavin and J. E. Smith (1978). "Age-related changes in percentage of fiber types and mean fiber diameters of the ovine quadriceps muscles." American Journal of Veterinary Research **39**(8): 1297-1302.

White, N. A., M. D. McGavin and J. E. Smith (1978). "Age-related changes in percentage of fiber types and mean fiber diameters of the ovine quadriceps muscles." American Journal of Veterinary Research **39**(8): 1297-1302.

Williams, S., T. Frye, D. M. Schaefer, K. K. Scheller and Q. Liu (1992). "Vitamin E." Meat International **3**: 853.

Winstanley, M. A. (1979). "The colour of meat." Nutrition & Food Science **79**(6): 5-8.

Wittenberg, J. B. (1970). "Myoglobin-facilitated oxygen diffusion: role of myoglobin in oxygen entry into muscle." Physiological Reviews **50**(4): 559-636.

Wittenberg, J. B. and B. A. Wittenberg (2003). "Myoglobin function reassessed." Journal of Experimental Biology **206**(12): 2011-2020.

Wolcott, M. L., D. J. Johnston, S. A. Barwick, C. L. Iker, J. M. Thompson and H. M. Burrow (2009). "Genetics of meat quality and carcass traits and the impact of tenderstretching in two tropical beef genotypes." Animal Production Science **49**(6): 383-398.

Wolf, R., D. Wolf and V. Ruocco (1998). "Vitamin E: the radical protector." Journal of the European Academy of Dermatology and Venereology **10**(2): 103-117.

Wood, J., R. Richardson, N. Scollan, A. Hopkins, R. Dunn, H. Buller and F. Whittington (2007). Quality of meat from biodiverse grassland. Occasional symposium - British Grassland Society.

Wulf, D. M., J. B. Morgan, S. K. Sanders, J. D. Tatum, G. C. Smith and S. Williams (1995). "Effects of dietary supplementation of vitamin E on storage and caselife properties of lamb retail cuts." J Anim Sci **73**(2): 399-405.

Yancey, J. and D. Kropf (2008). "Instrumental reflectance values of fresh pork are dependant on aperture size." Meat science **79**(4): 734-739.

Yin, M. C., C. Faustman, J. W. Riesen and S. N. Williams (1993). "α-Tocopherol and Ascorbate Delay Oxymyoglobin and Phospholipid Oxidation In Vitro." Journal of Food Science **58**(6): 1273-1276.

Yusa, K. and K. Shikama (1987). "Oxidation of oxymyoglobin to metmyoglobin with hydrogen peroxide: involvement of ferryl intermediate." Biochemistry **26**(21): 6684-6688.